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SULFAMERAZINE THERAPY IN EXPERIMENTAL CECAL COCCIDIOSIS OF CHICKENS

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Levine (1939, 1940, 1941) tested sulfanilamide, sulfapyridine, and sulfaguanidine for the control of *Eimeria* infections in chickens and found that each of these sulfonamides had considerable coccidiostatic effect on several species of coccidia, but only sulfaguanidine had a marked inhibitory effect on infections with *E. tenella* and *E. necatrix*. He found, moreover, that the feeding of sulfaguanidine had no curative effect on chickens already infected with either of the two species named. Farr and Allen (1942) also tested sulfaguanidine against *E. tenella* infections in chickens and found that it had effective prophylactic properties, but that it was of little value as a treatment when administered after the appearance of blood in the droppings. Horton-Smith and Taylor (1942, 1943), working with sulfadiazine and sulfamethazine, reported favorable results from the use of these drugs against cecal coccidiosis when treatment was delayed as late as 72 hours and 96 hours, respectively, after experimental inoculation. Sulfadiazine was administered in the mash and sulfamethazine was given either in one-half gram daily doses, or in saturated aqueous solution as a substitute for the drinking water. Hawkins (1943, 1944) reported that sulfamethazine showed considerable promise as a therapeutic agent for cecal coccidiosis. Swales (1944), reported that sulfamerazine had a definite curative effect on *E. tenella* infections even after intestinal hemorrhage had begun.

The present paper contains data on the use of sulfamerazine both as a prophylactic and curative agent for cecal coccidiosis of chickens.

EXPERIMENTAL PROCEDURE

The Rhode Island Red chickens used in these tests were obtained as day-old chicks from the poultry section of the Animal Husbandry Division, Bureau of Animal Industry, Agricultural Research Center, Beltsville, Md. They were immediately placed in electrically heated batteries and extreme care was taken to prevent them from acquiring an extraneous infection prior to experimental use. When removed from the batteries for use in an experiment, the birds were transferred to sterile all-metal brooders or cages. The brooders and cages were equipped with wire floors and with feeders and waterers that were attached to the outside to avoid contamination of the feed and water by the droppings. Newspapers were spread on the metal trays beneath the wire floors to facilitate the removal of the droppings

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and were changed daily. The infective oöcysts of *Eimeria tenella* used to inoculate the birds were prepared as follows: Scrapings containing large numbers of oöcysts from the cecal walls of experimentally infected birds were placed in a 2.5 per cent solution of potassium dichromate where the oöcysts sporulated. The oöcysts were then washed in distilled water, and stored in a refrigerator for use when needed. The birds were inoculated by introducing the sporulated oöcysts into the crop by means of a graduated pipette.

Each of the tests was set up and carried out at different times under essentially identical conditions. Where there are exceptions, these are mentioned at the proper places in the text. The birds were weighed and distributed in groups of equal number and of approximately equal weight. Those groups of birds which were to receive oöcysts were inoculated on the same day so that the infection in each of the groups would be as nearly comparable as possible. Each of the treated groups of birds received medicated mash for seven days and then was placed on regular mash for an additional seven days. The number and designations of all groups of birds in each experiment and the day on which treatment was started, with the single exception of the uninoculated-treated group, are given in Table 1. This latter group in experiment 1 was started on the medicated mash on the second day of the test, while in experiments 2 and 3 the treatment period began on the first day of the test. All the birds were weighed every other day except Sundays. The surviving birds were killed and autopsied at the end of the second week of each test.

Wehr and Farr (1945) found, in their work with sulfaguanidine, that the two groups on treatment four and five days after inoculation suffered no recurrence of the disease during the week following cessation of treatment. In the present studies, therefore, these two groups were not held a full seven days after withdrawal of the medicated mash.

In the text which follows, the designation for each group shown in the table will be used.

RESULTS AND DISCUSSION

With respect to the time of appearance and amounts of blood passed by the inoculated groups of birds, the effect on the course of infection of *E. tenella* in birds treated with sulfamerazine is similar to that of those treated with sulfaguanidine (1945).

In test 1, the 1–T (treated one day before inoculation), T–0 (treated on the day of inoculation), T–1 (treated one day after inoculation) and T–2 (treated two days after inoculation) groups of birds passed no blood during the time the drug was being administered while the T–3 (treated three days after inoculation), T–5 (treated five days after inoculation) and IC (inoculated control) groups passed considerable blood during the first week after inoculation. However, during the next week, blood was present in the droppings of the T–0 and T–1 groups on the fifth day, of the 1–T, T–0 and T–1 groups on the sixth day and of the 1–T group on the seventh day after withdrawal of the medicated mash. One chicken of the T–1 group died on the fifth day after cessation of treatment. One of its wings was gangrenous, and there was a trace of blood in the cecal contents. This was the only bird involved in the four experiments that died from a cause other than coccidiosis. The mean weight gain of the UTC (uninoculated-treated control) group for the period covering the experiment was considerably less than that of the UUC

TABLE 1.—*Effect of sulfamerazine on the course of infection with* E. tenella. *Each treated group received the drug for seven days*

Test No.	Age of birds at start of test	Concentration of drug in feed	Groups	Number birds per group	Number oöcysts administered	Mortality		Mean weight gains of survivors	Hemorrhage		No. of surviving birds with gross lesions of *E. tenella*		
						No. deaths	Per cent		1st week	2nd week	None	Slight	Marked
	Days	*Per cent*						*Grams*					
1	45	1	1–T Treated 1 day before inoc.	7	138,000	0	..	167	0	++‡	..	..	7
		1	T–0 " on day of "		"	0	..	176	0	++	..	..	7
		1	T–1 " 1 day after "		"	1*	14	200	0	+	..	1	5
		1	T–2 " 2 days " "		"	0	..	157	0	0	2	4	1
		1	T–3 " 3 days " "		"	0	..	171	++	0	1	4	2
		1	T–5 " 5 days " "		"	1	14	60	+++	0	..	1	2
		None	IC Inoculated controls		"	4	57	139	+++	0	..	1	2
		1	UTC Uninoculated treated controls		0	0	..	114	0	0	7	..	..
		None	UUC " untreated "		0	0	..	269	0	0	7	..	..
2	14	1/4	1–T Treated 1 day before inoc.	25	52,500	0	..	99	+	+	..	7	18
		1/4	T–0 " on day of "		"	0	..	110	+	+	3	11	11
		1/4	T–1 " 1 day after "		"	0	..	109	+	+	3	5	17
		1/4	T–2 " 2 days " "		"	0	..	119	+	0	1	7	17
		1/4	T–3 " 3 days " "		"	1	4	103	++	0	2	7	15
		1/4	T–4 " 4 days " "		"	5	20	94	++	+	2	4	14
		1/4	T–5 " 5 days " "		"	9	36	77	+++	0	2	4	10
		None	IC Inoculated controls		"	15	60	99	+++	+	..	3	7
		1/4	UTC Uninoculated treated controls		0	0	..	117	0	0	25	..	..
		None	UUC " untreated "		0	0	..	131	0	0	25	..	..
3	14	1/4	1–T Treated 1 day before inoc.	23	52,600	0	..	120	+	+	1	9	13
		1/4	T–0 " on day of "		"	0	..	116	+	+	6	8	9
		1/4	T–1 " 1 day after "		"	0	..	113	++	+	6	7	10
		1/4	T–2 " 2 days " "		"	2	8	99	++	0	6	4	11
		1/4	T–3 " 3 days " "		"	5	21	97	+++	0	5	5	8
		1/4	T–4 " 4 days " "		"	4	17	100	+++	0	10	6	3
		1/4	T–5 " 5 days " "		"	8	34	87	+++	0	1	3	11
		None	IC Inoculated controls		"	8	34	104	+++	0	3	2	10
		1/4	UTC Uninoculated treated controls		0	0	..	119	0	0	23	..	..
		None	UUC " untreated "		0	0	..	130	0	0	23	..	..
4	26	1	1–T Treated 1 day before inoc.	10	102,000	1†	10	136	0	+++	..	2	7
		1	T–0 " on day of "		"	0	..	108	0	++	..	1	9
		1	T–1 " 1 day after "		"	0	..	128	0	+	1	4	5
		None	IC Inoculated controls		"	6	60	116	+++	+	..	..	4
		None	UUC Uninoculated untreated controls		0	0	..	175	0	0	10	..	..

* This bird died of a wing infection.
† This bird died of cecal coccidiosis on the 5th day after removal of the medicated mash.
‡ Number of + indicates degree.

(uninoculated-untreated control) group for the same period. At autopsy, necrotic areas were present in the spleen and livers of half the treated birds. That these necrotic areas were probably due to the toxic effects of the drug is evidenced by their absence in the spleen and livers of any of the untreated birds. The drug, in spite of indications of toxicity, afforded the birds some protection against the disease, as only 1 of the 42 treated-inoculated birds died of coccidiosis, whereas 4 of the 7 inoculated controls died of this disease.

In tests 2 and 3, a mash containing only one-fourth of the amount of sulfamerazine fed in test 1 was used as the medicament. In both of these tests all of the inoculated groups of birds passed blood while on treatment. In test 2, blood appeared first in the droppings of the T–2, T–3, T–4, T–5 and the IC groups on the fifth day after inoculation; blood was not observed in the droppings of 1–T, T–0 and T–1 groups until one day later. The amounts of blood passed were relatively small in the groups placed on treatment on or before the second day after inoculation. Blood continued to appear in the droppings of the 1–T, T–5 and IC groups on the seventh day after inoculation, and in the 1–T and IC groups on the eighth day after inoculation. No blood appeared in the droppings of the T–0, T–1 and T–4 (treated four days after inoculation) groups on the seventh day after inoculation but reappeared on the eighth day. The reappearance of blood in the droppings of T–0, T–1 and T–4 groups after a lapse of one full day is probably not a recurrence due to the resumption of development of the coccidia, but merely a temporary cessation due to obstruction of the cecal openings or to some other cause. On the other hand, in experiment 3, traces of blood appeared in the droppings of the 1–T and T–0 groups on the fifth day after the removal of the medicated mash and in the droppings of the T–1 group on the sixth day. At autopsy, 10 of the treated birds of tests 2 and 3 of the treated birds of test 3 had necrotic areas in the spleen; only one treated bird of each of these two tests had liver lesions.

In test 2 there seemed to be a closer correlation between the time treatment was begun and the number of deaths than in test 3. This correlation was in the form of a progressive decrease in the percentage of deaths as the time between inoculation and treatment decreased. No deaths occurred in the birds of test 2 which were placed on treatment either one day before inoculation, at the time of inoculation, or one or two days after inoculation. Of the remaining birds, 96 per cent of those treated 3 days and 80 per cent of those treated 4 days after inoculation survived; 64 per cent of those treated 5 days and 40 per cent of the inoculated controls, respectively, survived. In test 3, there were no deaths among the birds placed on treatment either 1 day before, at time of, or 1 day after inoculation. Ninety-two per cent of the birds that were subjected to treatment 2 days after inoculation, 79 per cent of those treated 3 days, 83 per cent of those treated 4 days, and 66 per cent of those treated 5 days after inoculation survived; 66 per cent of the inoculated controls survived. It appears, therefore, that no great claims can be made for one-fourth per cent concentration of sulfamerazine as a control measure, when administered to birds later than the second day after inoculation. That the drug is apparently toxic even when fed in a concentration as low as one-fourth per cent is indicated by the lower mean weight gains of all the treated groups as compared with those of the UUC groups, and by the presence of spleen and liver lesions in some birds of both tests 2 and 3. The relatively greater number of deaths among the treated groups

and the smaller number in the inoculated controls of test 3 seem to indicate that these birds derived less benefit from the treatment than those of test 2.

The delayed appearance of blood in the droppings of the birds of groups 1–T, T–0 and T–1 of experiment 1, raised the question as to whether the birds had accidentally become reinfected or whether the drug had exerted a coccidiostatic effect. Test 4 was set up to throw some light on this point. Except for the number of groups of birds included, this experiment was performed in a manner similar to experiment 1. In this test each group of birds was transferred to a sterilized cage on the day the medicated mash was removed and three of the UUC birds were placed in the cage with the infected birds. The inoculated controls passed copious amounts of blood on the fifth and sixth days after inoculation and smaller amounts on the seventh day, while the treated birds passed no blood during this period. Six of the 10 inoculated controls died of cecal coccidiosis. The members of the 1–T group passed considerable blood on the fourth and fifth days after the removal of the medicated mash and were obviously sick at this time, one bird dying of cecal coccidiosis on the fifth day. The birds of the T–0 and T–1 groups also passed blood on the fourth and fifth days after cessation of treatment but were not nearly so sick as those of the 1–T group. At necropsy, all of the treated birds, except one, showed macroscopic lesions of cecal coccidiosis. The UUC birds appeared to be in good condition throughout the test and when examined post mortem, the ceca of these birds appeared normal and no coccidia were found in smears of the cecal contents. As a further check, the cecal contents of five of these birds were examined by the sugar solution flotation method. No oöcysts were found in four of the birds; a few oöcysts, less than 10, were recovered from the cecal contents of the fifth bird.

Since the UUC birds showed no clinical symptoms and no macroscopic lesions of cecal coccidiosis, it is clear that there was little possibility that reinfection occurred.

In case reinfection did take place, as may be indicated by the few oöcysts found in one of the UUC birds, it could not have produced the conditions observed in the treated birds.

The results of this experiment strongly indicate that the drug prevented the development of coccidia. That this may be the correct interpretation is shown by a study of the group which was placed on treatment one day before inoculation and, therefore, probably received the greatest amount of protection from the drug. The characteristic symptoms of cecal coccidiosis did not appear in this group until four days after the cessation of treatment and the disease was severe enough to cause the death of one bird on the fifth day.

With respect to behavior of the disease in chickens as influenced by the feeding of sulfamerazine mash, the results of experiments 1 and 3 substantiate those of experiment 4, except that during the second week the blood appeared one or two days later in the droppings of birds of experiments 1 and 3. In experiment 2, the presence of blood in the droppings of the birds during the second week did not seem to bear any relationship to the time of withdrawal of the medicated mash.

In-so-far as it is possible to ascertain from a clinical study of the living infected bird, the effect of sulfamerazine treatment on the course of infection with *E. tenella* is apparently similar to that of sulfaguanidine. However, the present studies show that the feeding of sulfamerazine was associated with much lower mean weight gains of the UTC groups as compared with those of the UUC groups, and the

presence in several of the birds of liver and spleen lesions. Wehr and Farr (1945) did not encounter these ill effects in chickens fed sulfaguanidine.

From a consideration of the life cycle of *E. tenella,* a species requiring 7 days to complete its development within the avian host, it seems that the two drugs in question exert a static effect on the development of the coccidial parasites.

SUMMARY AND CONCLUSIONS

1. Experiments were conducted with 593 chickens to determine the effect of sulfamerazine on the course of infection with *Eimeria tenella.*

2. Sulfamerazine administered in the mash in a concentration of one per cent materially reduced mortality from cecal coccidiosis. This percentage of the drug was toxic as indicated by the presence of spleen and liver lesions and of retarded weight gains.

3. One-fourth per cent sulfamerazine mash was an effective prophylactic agent for cecal coccidiosis and also was of benefit as a treatment when fed within two days after inoculation. This percentage was considerably less toxic than one per cent but an occasional bird showed spleen and liver lesions; some loss in weight gains was noted.

4. When fed before or within one day after inoculation one per cent sulfamerazine was markedly coccidiostatic as long as it was being administered. Within four to six days after cessation of this treatment clinical coccidiosis developed.

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EFFECT OF SULFAGUANIDINE ON THE COURSE OF INFECTION IN CHICKENS WITH *EIMERIA TENELLA*

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Of the sulfonamides which have been tested experimentally against avian coccidiosis, sulfaguanidine has shown considerable promise as a prophylactic in this disease. By a series of tests in which this drug was administered in the mash, Levine (1941) found that sulfaguanidine prevented infection of birds with certain of the species of coccidia which complete their development in the small intestine and markedly reduced the severity of infection with *Eimeria tenella* and *E. necatrix,* which complete their development in the ceca. The institution of sulfaguanidine treatment three days after experimental inoculation did not, according to Levine, alter the course of infection with *E. tenella* and *E. necatrix.* Farr and Allen (1942) reported that birds which had received sulfaguanidine in mash, in either 1 or 2 per cent concentration, for three days before and for nine days after inoculation with *E. tenella* showed no symptoms or severe lesions. They reported, moreover, that birds to which either 3 or 5 per cent sulfaguanidine in the mash was given for one week beginning at the time of the appearance of blood in the droppings were not significantly benefited, although oöcyst production was sharply reduced.

The purpose of the studies reported in this paper was to ascertain the effect, on the course of infection with *Eimeria tenella,* of sulfaguanidine in mash when administered for 7 consecutive days, beginning one day prior to, on the day of, and one, two, three, four and five days, respectively, after inoculation.

MATERIALS AND METHODS

The birds used in these tests were obtained as day-old chicks from the poultry section of the Animal Husbandry Division, Beltsville Research Center, Beltsville, Md. When 16 days old, the chicks were weighed, banded, and then sorted on the basis of their weights, into the required number of groups, the lightest and heaviest birds being discarded so as to make for greater uniformity among the groups. Each group was composed of an equal number of birds, the total weights of the birds of each group being very close. A wire-bottomed brooder unit, which was equipped with feed and water containers attached to the outside of the unit, served as a confinement unit for each group of birds. Newspapers were spread over the metal trays beneath the wire floors to catch the droppings and to facilitate their removal each day. Two days were allowed for the birds to become accustomed to their new surroundings and to permit the return to normal feeding before the experiment was begun. It was possible sometimes within this period to detect birds which, for one reason or another, might detract from the uniformity of the group as a whole and thereby influence the results. These birds were eliminated, therefore, from the experiment. The total number of birds for the different groups of each test as listed in Table 1 is different because of the final check-up and elimination.

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TABLE 1.—*Effect on the course of infection with* Eimeria tenella *of sulfaguanidine. Each treated group received the drug for 7 consecutive days*

Exp. No.	Age of birds at start of exp.	Conc. of drug in mash	Groups	Number birds per group	Number oöcysts given per bird	Haemorrhage		Mean wt. gains of survivors	Mortality		Surviving birds having—		
						1st week	2nd week		No.	Per cent	No lesions	Slight lesions	Marked lesions
		Per cent						*Grams*			*No.*	*No.*	*No.*
1	16	0.5	1–T, Treatment began 1 day before inoculation	20†	68,000	+*	+	110.8	1	5	0	5	11
			T–1, " " " " after "	19	"	+	+	99.4	4	21	2	2	11
			T–2, " " 2 days " "	19†	"	+++	..	93.0	6	31	1	2	9
			T–3, " " 3 " " "	20†	"	+++	..	95.8	12	60	0	1	6
			T–4, " " 4 " " "	20	"	+++	..	59.0	17	85	0	0	3
			T–5, " " 5 " " "	20	"	+++	..	69.0	17	85	1	1	1
			IC, Inoculated Controls	20	"	+++	..	80.5	18	90	0	1	1
			UTC, Uninoculated-Treated Controls	18	0	0	..	129.7	0	...	18	0	0
			UUC, Uninoculated-Untreated Controls	18	0	0	..	133.9	0	...	18	0	0
2	16	1.2	1–T, Treatment began 1 day before inoculation	20	101,000	+	++	155.0	0	...	12	6	2
			T–0, " " on same day as inoculation	20†	"	+	+	154.3	0	...	12	7	0
			T–1, " " 1 day after inoculation	21	"	+	+	153.9	0	...	2	18	1
			T–2, " " 2 days " "	22	"	++	..	136.6	1	4.5	1	9	11
			T–3, " " 3 " " "	22	"	+++	..	121.2	3	13.6	2	4	13
			T–4, " " 4 " " "	21	"	+++	..	106.8	10	47.6	0	1	10
			T–5, " " 5 " " "	22	"	+++	..	103.3	18	81.8	1	1	2
			IC, Inoculated Controls	22	"	+++	..	87.5	20	90.9	0	0	2
			UTC, Uninoculated-Treated Controls	22	0	0	..	169.0	0	...	22	0	0
			UUC, Uninoculated-Untreated Controls	22	0	0	..	158.9	0	...	22	0	0

* Plus sign indicates relative amount of blood passed.
† Not all of surviving birds examined.

The care of the birds was assigned to one person who kept feed and clean water before them all the time and removed the droppings daily.

Rhode Island Red chicks infected with *E. tenella* were used as a source of oöcysts. The birds were inoculated with just enough sporulated oöcysts to produce at the most only traces of blood. Such lightly infected birds usually showed very little disturbance from the infection and yielded large numbers of oöcysts. All donor birds were sacrificed on the seventh or eighth day after inoculation and the cecal pouches removed. The scrapings of the ceca containing the unsporulated oöcysts were immediately placed in a 2.5 per cent potassium dichromate solution and allowed to remain at room temperatures until the oöcysts sporulated. The oöcysts were then washed repeatedly in water. The test birds were infected by introducing the oöcysts into the crops with a graduated pipette.

For measuring the oöcyst output, the dilution method employed by Farr and Allen (1942) was used. Oöcysts were recorded as oöcysts in thousands per gram of feces. While the method used for detecting the presence of oöcysts in the droppings lacked the refinement and accuracy of other methods, it had the advantages of simplicity and speed that are required when numerous samples have to be examined over a short period.

Each experiment was conducted independently and at different times. The setup of experiment 2 differed from that of experiment 1 in that (1) a higher percentage of the drug was fed in the mash; (2) a greater number of oöcysts was administered to each bird; and (3) an extra group, treated at the time of inoculation, was added.

Sulfaguanidine in mash was fed continuously for seven days. Nonmedicated mash was substituted for the medicated mash for the remaining period of the experiment which, in the case of experiment 1, was 6 days and, in the case of experiment 2, 7 days. The purpose of holding the treated birds for one week after the withdrawal of the medicated mash was to ascertain if any interference in the course of the infection might result from the medication.

Observations on the development of possible toxic effects of the drug, the time of appearance of blood in the droppings of each of the inoculated groups, and the general behavior of the birds, were noted daily. Dead birds were removed daily from the brooder units and promptly examined for signs of toxicity and for presence of coccidia. The cause of death, if ascertained, was recorded.

For convenience, symbols, as explained in Table 1, are used to designate particular groups within an experiment.

RESULTS AND DISCUSSION

The results of two experiments involving the administration of sulfaguanidine in mash in two different concentrations to chickens before and after experimental inoculation with *Eimeria tenella* are presented in summarized form in Table 1. Table 2 shows the oöcyst output in thousands per gram of feces of the IC (inoculated controls), 1–T (treated 1 day before inoculation), T–0 (treated on same day as inoculated), T–1 (treated 1 day after inoculation), and T–2 (treated 2 days after inoculation) groups of experiment 2 over a period of successive days after the withdrawal of the medicated mash. Irrespective of the differences in the structural setup of the two experiments, the results are, except in degree, similar.

The data as presented permit certain broad conclusions: (1) that the greatest benefit from sulfaguanidine treatment was derived by the 1–T, T–0, T–1, T–2, and T–3 (treated 3 days after inoculation) groups; (2) that the drug was apparently responsible for the reduced amounts of blood passed by the 1–T, T–0 and T–1 groups during the period of treatment; and (3) that the drug apparently exerted a coccidiostatic effect was demonstrated by the reappearance of blood in the droppings of the 1–T, T–0, and T–1 groups, as well as by the gradual increase in oöcyst output, during the week after the withdrawal of the medicated mash; (4) that the reappearance of blood in the droppings of the 1–T, T–0, and T–1 groups during the week following the withdrawal of the medicated mash was probably not due to reinfection of the birds. This has been satisfactorily demonstrated, it is believed, in our paper on sulfamerazine therapy (1945).

The lower mortality percentages and the greater weight gains of the 1–T, T–0, T–1, T–2 and T–3 groups justify the first conclusion. Little or no benefit from sulfaguanidine therapy was derived by either the birds of experiment 1 which were placed

TABLE 2.—*Oöcyst output of the IC, 1–T, T–0, T–1 and T–2 groups of experiment 2 in thousands of oöcysts per gram of droppings*

Number of days after inoculation	IC	1–T	T–0	T–1	T–2
7	1,695	0	...	...	..
8	2,520	0	0	...	..
9	256	50	0	...	..
10	115	95	3	0	..
11	83	186	70	23	0
12	66	803	63	76	0
13	63	420	440	90	3
14	70	406	550	442	10
15	53	396	353	316	63
16	30	336	310	153	73
17	3	...	153	126	23
18	10	...	...	50	6

on medicated mash later than the third day or by the groups of birds of experiment 2 which were started on treatment later than the fourth day after infection, as the percentage of mortality in each of these groups was much higher than that of any of the other groups. The absence of any deaths in the groups 1–T, T–0 and T–1 and comparatively few deaths in the groups T–2, T–3, and T–4 (treated 4 days after inoculation) of experiment 2 testify to the greater severity of the infection in corresponding groups of experiment 1.

In an effort to find a cause for the greater severity of the infection in the treated birds of experiment 1, the principal points to be considered are the numbers of oöcysts administered and the concentration of the drug fed to the birds of each experiment. It is apparent that the number of oöcysts administered had no bearing on the intensity of the infection in the birds of the two experiments because (1) a larger number of oöcysts were administered to the birds of experiment 2, and (2) the percentage of mortality in the IC group of birds of each experiment was almost identical. This leaves, therefore, for consideration only the concentration of the drug.

The fact that the lower concentration of the drug in the mash fed to the birds of experiment 1 apparently afforded less protection than the higher concentration given to the birds of experiment 2 is based on the following observations: (1) the 1–T, T–0 and T–1 groups of experiment 2 survived the infection *in toto,* whereas the 1–T and T–1 groups of experiment 1 (the T–0 group was not represented) had

1 and 4 deaths, respectively; (2) the T–2, T–3, and T–4 groups of experiment 2 sustained fewer losses than these same groups of experiment 1; (3) there was a larger percentage of surviving birds with no gross lesions of *Eimeria tenella* in experiment 2.

Part of the data presented in Table 1 is plotted on semi-logarithmic paper and shown as Fig. 1. These data show that when treatment was instituted on or before one day after experimental inoculation, there were no deaths as in experiment 2 or

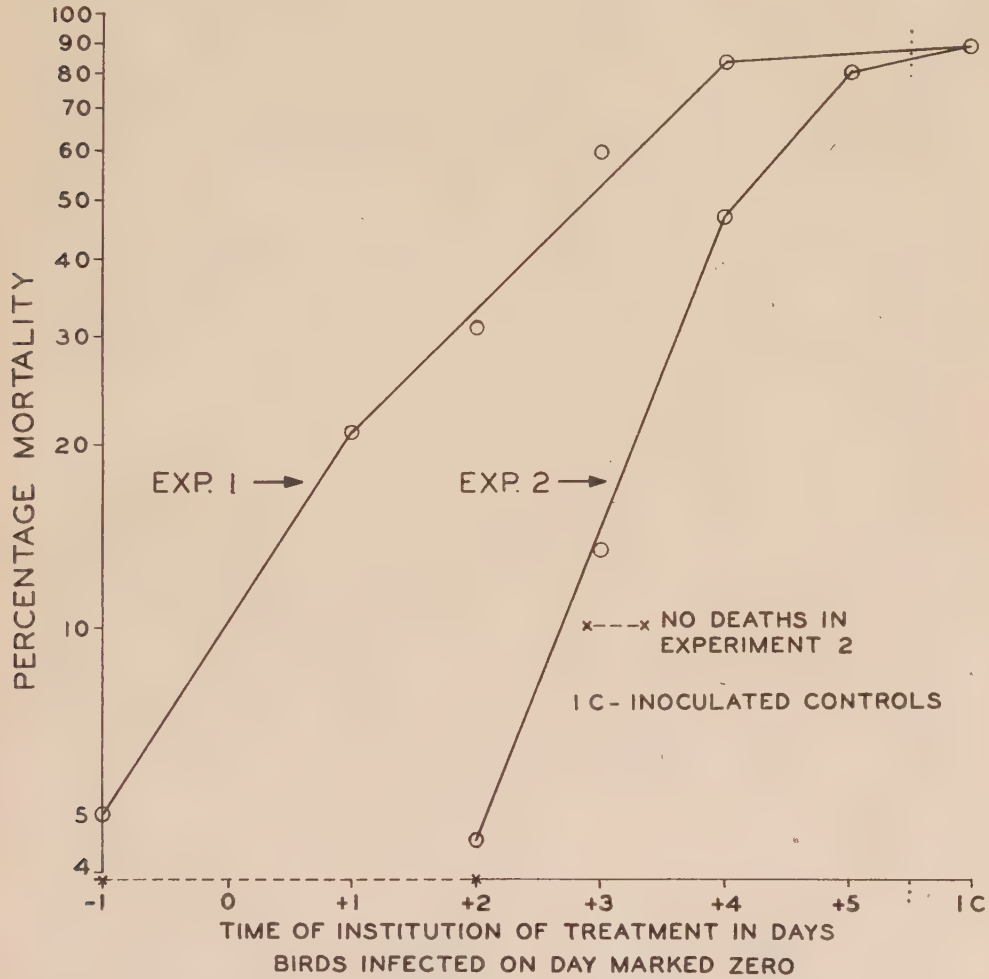

FIG. 1. Graph showing relationship between increase in mortality rate and time of treatment.

relatively few as in experiment 1. However, when treatment was instituted later than one day after experimental inoculation, the death rate increased with delay in drug treatment. In those groups in which the institution of treatment was delayed until the fourth day as in experiment 1 and the fifth day as in experiment 2 after inoculation, the increase in the death rates did not differ materially from those of the inoculated controls. Closer scrutiny of the chart shows that the death rate increased most rapidly during the period from plus 1 to plus 4 days in experiment 1

and from plus 2 to plus 4 in experiment 2. Furthermore, since the increase in death rate is represented on the logarithmic chart as a linear relationship to delay in inception of treatment, this would indicate, because of the nature of this type of chart, that each day in delay of institution of treatment is associated with a regular increase in death rate within these periods.

The amounts of blood passed by the birds of the two experiments were similar and served as evidence that the treatment, at least in certain of the groups, in some way interfered with the normal course of the infection. On the fifth day after experimental infection, blood was present in the droppings of all the inoculated groups of experiment 1, in small amounts in the 1–T and T–1 groups, and in considerably greater amounts in the other groups. Blood continued to be passed in small amounts in the droppings of the birds of all groups through the seventh day. The 1–T and the T–1 groups were the only birds to pass blood in the droppings during the second week of infection. Small amounts of blood were observed in the droppings of the 1–T group on the sixth day and of the T–1 on the fourth day after the withdrawal of the medicated mash. On the fourth day after inoculation, small amounts of blood were seen in the droppings of the IC, T–3, T–4, and T–5 (treated 5 days after inoculation) groups of experiment 2. However, on the fifth day, traces of blood appeared in the droppings of the 1–T, T–0, T–1 and T–2 groups, while larger amounts appeared in the droppings of the IC, T–3, T–4 and T–5 groups. Blood in small amounts was passed with the droppings of 1–T, T–0 and T–1 groups on the sixth day after inoculation and in larger amounts in all the other groups; none was observed in any of the groups on the seventh day. Traces of blood were observed in the droppings of only the 1–T, T–0 and T–1 groups on the fourth and fifth days after the discontinuance of feeding of medicated mash, of the T–0 group on the sixth day, and of the 1–T group on the seventh and eighth days.

The delayed appearance of blood and the passage of smaller amounts of blood in the droppings of the 1–T, T–0 and T–1 groups of experiment 2, and the reappearance of blood in the droppings of the above groups during the week following the withdrawal of the medicated mash are unquestionably associated with treatment and are indicative of a static action of the drug against coccidia. A similar phenomenon was observed by Farr and Wehr (1945) as a result of feeding sulfamerazine to chickens infected with *Eimeria tenella*. The gradual increase in oöcyst output of the 1–T, T–0, T–1 and T–2 groups and a steady decline in the oöcyst output of the IC groups following withdrawal of the medicated mash demonstrate further the possible static action of the drug.

Oöcyst counts on those groups which were placed on medicated mash later than the second day after experimental inoculation are not included in Table 2 because those birds did not appear to be benefited to the same degree as those placed on sulfaguanidine mash earlier. A study of the table will show that the peak of oöcyst production in the IC group was reached on the eighth day after experimental inoculation, with a steady decline, thereafter, except for two days when there was a slight rise, until the discontinuance of oöcyst counts on the eighteenth day after infection. In contrast to this, the treated groups showed no oöcysts for the first day or two after the removal of the medicated mash, but there was a rather rapid increase in the oöcyst output by these groups for the next few days, the peak being reached on the sixth and seventh days after the withdrawal of the medicated mash; then a steady

decline took place. It should be noted that as the period between inoculation and treatment increased there was a progressive decrease in the total number of oöcysts produced, with the lowest production in the group placed on medicated mash two days after inoculation.

The results of the present studies, in so far as they relate to the effect of the drug on the oöcyst output of the treated birds are in close agreement with those reported by Levine (1939, 1940). This investigator demonstrated a suppression of the oöcyst output in birds infected with *Eimeria praecox, E. maxima, E. mitis,* or *E. hagani* as the result of the feeding of 0.3-gm dose of sulfanilamide daily. However, within one or two days after the cessation of treatment with this drug, the number of oöcysts in the droppings began to increase until the peak was reached on the third or fourth day. Levine found also that while chickens infected with *E. praecox, E. mitis,* or *E. hagani* were being treated with 0.7 per cent sulfapyridine mash, the oöcyst output was relatively small. But when the medicated mash was removed "a gradual increase in the oöcyst discharge occurred and peaks were reached five days later." In none of his experiments on the use of sulfaguanidine did Levine include groups of birds to which treatment was administered on all of the days included in the present studies. Therefore, no comparison of the results of Levine's work with sulfaguanidine and those presented herein can be made.

SUMMARY AND CONCLUSIONS

1. Experiments were conducted with 388 birds to determine the effect of sulfaguanadine on the course of infection with *E. tenella.*

2. The feeding of 0.5 per cent sulfaguanidine mash for 7 consecutive days decidedly benefited young birds when treatment was begun 1 day before or within 2 days after experimental infection.

3. The feeding of mash containing 1.2 per cent sulfaguanidine for 7 consecutive days prevented deaths among groups of birds placed on treatment one day prior to, on the day of, and one day after inoculation. The administration of this mash as late as the third day after infection markedly reduced mortality.

4. The results of the present studies apparently demonstrate a coccidiostatic action of sulfaguanidine.

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VARIATIONS IN RESPONSE TO FILARIFORM LARVAE OF *ANCYLOSTOMA CANINUM* IN THE SKIN OF MAN

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INTRODUCTION

Reports on the reaction of the skin of man to penetration by filariform larvae of *Ancylostoma caninum* (Ercolani, 1859) Hall, 1913, have been in conflict on several points, particularly with regard to the subsequent behavior and fate of the worms. White and Dove (1929) described a transient papular dermatitis, clearly distinct from the clinical appearance of burrows that follow penetration by larvae of *Ancylostoma braziliense* de Fairo, 1910. Heydon (1929) and Fülleborn (1930), however, reported a more prolonged reaction, apparently produced by deep penetration and an ensuing aimless migration or "creeping eruption" of the parasites through the superficial tissues. These reports raised the following questions: (1) are there separate strains of *A. caninum,* possessing variable abilities to invade the skin of man? or (2) does the human subject vary constitutionally in his ability to arrest the invasion of a parasite which may be considered uniform within its species?

The following work, carried out with a single culture of *A. caninum* larvae, presents evidence which would answer the latter question in the affirmative.

MATERIALS AND METHODS

Two volunteers, one of whom had a past medical history of multiple allergies, infected themselves on the volar surface of their right forearms, each with 1000 to 1500 *A. caninum* filariform larvae obtained from Dr. W. W. Cort, Johns Hopkins University. These larvae were secured from feces of laboratory-housed dogs known to harbor a pure culture of this species of worm. The larvae were washed until macroscopically clean, but no bacteriostatic agents were used.

The resultant infections were unexpectedly violent in character. Surgical consultation elicited the opinion that a bacterial cellulitis was superimposed on the helminthic process, microörganisms having penetrated the cutaneous sites of entry along with the larvae. Heydon (1929) remarked of one of his cases that, if he had been unaware of the true etiology, he would naturally have suspected a septic process.

The symptoms and progress of each subject are presented and compared below.

RESULTS

1. *Subject A.* No past allergic history.

A prickling sensation was noted during the time of application of the larvae. Within forty-five minutes the area was distinctly erythematous and itching had developed. The inflammation extended rapidly, accompanied by increasing pruritus, and in forty-eight hours virtually the entire anterior aspect of the forearm from wrist to elbow was involved. Urticarial lesions, some sinuous in outline, were present

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radially, while at the site of penetration numerous small papules were evident. The forearm felt tense and axillary lymph nodes were slightly tender.

On the third day the forearm was indurated and tender. Sulfadiazine, 4 grams daily, was instituted. During the next three days pain, induration and erythema began slowly to subside. Sulfadiazine dosage was then cut to 1 gram twice daily for the next four days.

By the twelfth day the forearm appeared and felt normal except for a few small areas where slight tension still existed. The subject had remained ambulatory throughout and had had no systemic reaction except for a slight elevation of temperature (less than one degree F.) on the second and third days. His subsequent reactions consisted of occasional transient pruritus and edema (urticarial wheals) at or

TABLE 1.—*Total and differential white blood cell counts*

Subject A—Non-allergic							
Day	WBC	Polys	Lymphs	Monos	Basos	% Eos	Eos/cmm
3	9,900	76	17	5	0	2	198
7	7,800	71	23	4	1	1	78
10	7,100	67	28	4	1	0	....
26	6,300	73	21	5	0	1	63
85	8,850	62	24	5	0	9	797
92	7,000	65	22	7	0	6	420
99	7,200	52	43	2	0	3	216
120	6,200	57	33	5	0	5	310
Subject B—Allergic							
Day	WBC	Polys	Lymphs	Monos	Basos	% Eos	Eos/cmm
8	8,700	71	24	1	0	4	348
10	12,400	75	18	0	0	7	868
12	8,900	68	20	8	0	4	356
29	12,800	62	12	0	0	26*	3,328
33	11,900	50	44	0	2	4	476
72	12,000	50	30	4	1	15	1,800
77	11,800	56	25	5	0	14	1,652
84	11,600	50	30	10	0	10	1,160
92	10,050	68	14	7	0	11	1,105
98	9,900	58	26	4	0	12	1,188
102	10,250	71	15	4	0	10	1,025
106	14,250	74	14	5	0	7	998
120	8,700	52	38	4	0	6	522
181	13,400	64	23	5	0	8	1,072
209	.....	78	12	4	0	6	....

* Sulfa rash ; onset of "flu."

near the site of inoculation. These episodes occurred at increasing intervals of time for several months, each one lasting a day or two. On the twenty-seventh day he had one such lesion excised in order to determine the presence or absence of a migrating larva as its cause. Serial sections of this tissue, fixed in Zenker's, showed no evidence of a parasite in the epidermis or corium, though some sections exhibited slight lymphoid and plasma cell infiltration around the papillae. The pathologist's diagnosis was "slight chronic inflammatory reaction."

Total and differential white blood cell counts made during the course of the infection are presented in Table 1 and plotted in Fig. 1. Other laboratory work consisted chiefly of repeated stool examination to search for *A. caninum* ova. Although there were no gastro-intestinal symptoms suggestive of the presence of adult worms, this species has been known rarely to reach the intestine of man and become mature (Manalang, 1925; Dove, 1932). Results of stool examinations are presented in Table 2.

2. *Subject B.* Past allergic history as follows. Allergic coryza (late summer

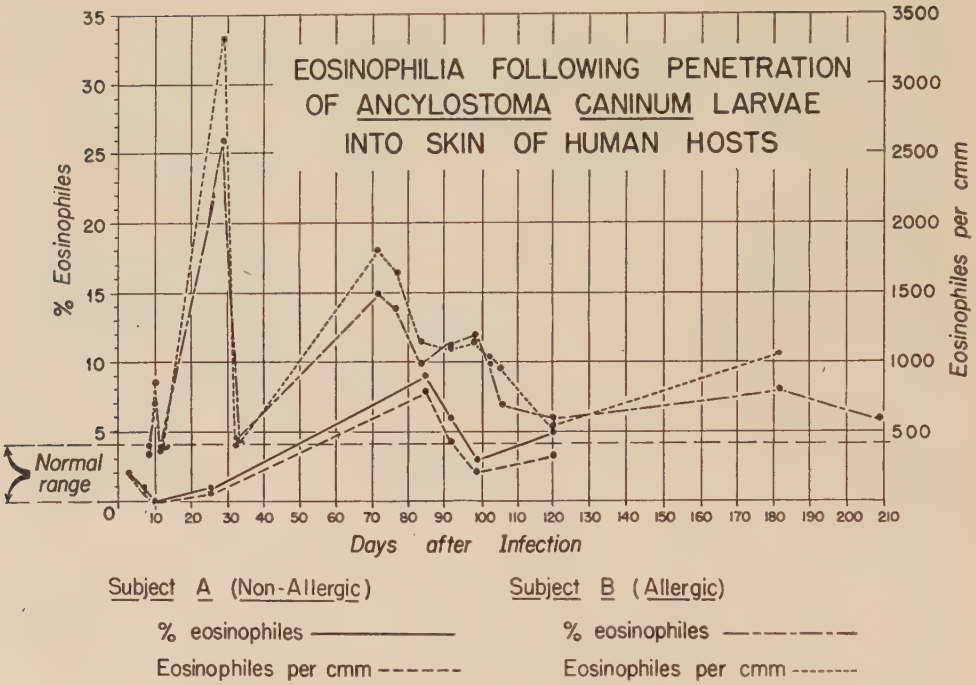

FIG. 1. Range of eosinophilia in allergic and non-allergic subjects following cutaneous penetration of infective *Ancylostoma caninum* larvae.

variety, eastern United States) from infancy, decreasing in early adult life. Chief allergen ragweed. No asthma. Food sensitivities: celery, parsley, egg, hazel-nut, carrot and many others to a less degree. Sensitive to horse and cat dander. Sensitive to atropine. Siblings and paternal lineage also allergic.

This subject had ascariasis twenty-three years previously. It cannot be said, however, whether or not he thus became sensitive to a nematode-type protein. The infection at that time was asymptomatic.

Following the application of *A. caninum* larvae he felt less immediate pruritus than the first subject, and the erythema was also milder. On the second day macules and papules were visible at the points of entry. This was followed on the third day by a spreading inflammation, slight itching and moderate superficial tenderness. The papules were becoming vesicular and the epitrochlear lymph node was palpable. The skin was tense, hot and dry. Sulfadiazine, 4 grams daily, was begun.

On the fourth day burrows were clearly evident. These appeared first as

TABLE 2.—*Stool examinations*

Day	Subject A	Subject B
3	Negative for helminths	....
19	Negative for helminths	....
30	*Endolimax nana*	....
31	*Endolimax nana*	....
37	.....	Negative
88	*Giardia lamblia*	....
89	*Giardia lamblia*	....
97	*G. lamblia* and *E. nana*	....
104	.....	Negative
105	*Giardia lamblia*	....
108	*Giardia lamblia*	....

vaguely outlined, slightly elevated, tortuous, erythematous lines but subsequently were marked by a succession of vesicles which ruptured and became crusted, each in turn.

From the fourth to the sixth day sulfadiazine was continued and the swelling receded slightly. On the seventh day, however, the subject experienced a sudden onset of deep induration of the entire forearm with severe aching pain throughout the thickness of the member. On admission to surgical service of the hospital he had the following symptoms: pitting edema of forearm with small vesicles; minimal signs of bacterial infection; palpable epitrochlear and axillary nodes; no pain in forearm with extension of fingers and wrist; temperature, pulse and respiration normal. Impression: cellulitis of right forearm.

The subject was placed on a routine in which sulfadiazine was continued. In addition wet boric dressings, with a heating pad, were applied constantly. Daily soaking in a hot tapwater whirlpool bath for about an hour was also instituted. Shelmire (1928) has suggested that heat be contraindicated in the therapy of creeping eruption due to its tendency to increase the activity and wandering of the larvae.

The surgeon's further notes were:

"9th day. Edema slowly subsiding. X-ray of forearm negative.

"19th day. Edema seems to be subsiding; however, the parasites appear to be active, migrating toward the tip of the digits.

"24th day. Skin rash; appears due to the sulfadiazine; this will be discontinued. Temperature, pulse and respiration normal. Whirlpool continued.

"30th day. Severe case of flu, temperature 102° F. No findings in chest. Bed care.

"34th day. Temperature, pulse and respiration normal. General condition good. Right arm OK.

"38th day. Discharged, cured. Final diagnosis: cellulitis, acute, suppurative, right forearm, severe—secondary to dog hookworm."

Other laboratory findings on Subject B during the period of hospitalization included negative blood Kahn reaction on 14th day, bleeding and clotting times 1 and 4 minutes respectively on 8th day, blood sulfadiazine levels of 8.9 and 5.7 on 8th and 10th days, respectively, negative urines on 10th and 12th days, and red blood values averaging 4,500,000 RBC and 80–85 per cent hemoglobin throughout the period of the experiment.

For the next several months the subject had numerous recurrences of symptoms, although none of these required further medical care. At times there were generalized or localized swellings; at others there were renewed burrowings. These episodes were most noticeable following active use of the right forearm (chopping, sawing, etc.). On the 53rd day a long burrow appeared on the ulnar side of the wrist. On the 59th day the dorsum of the hand was swollen. On the 69th day there was again swelling of the dorsum of the hand, followed on suceeding days by swelling first of the wrist (volar aspect) and then of the elbow (lateral aspect). These episodes were attended by erythema with central urticarial wheals of irregular serpentine shape but without definite burrows. Further symptoms—with swellings, burrows or both—occurred on the 76th, 78th, 102nd, 177th and 208th days, usually as a sequel of direct trauma to the arm or of an upper respiratory infection.

SEROLOGICAL STUDIES

Otto (1939) and Sarles (1938) have shown that precipitates form about filariform larvae immersed in hyperimmune serum of experimental animals, while serum from nonimmune hosts exhibits no such activity. Since the subjects of the present study had experienced massive infection, experiments were performed on both their sera on the 77th and 87th days. Living *A. caninum* filariform larvae were immersed in serum on glass slides under cover glasses sealed with vaseline. Controls were set up both in normal serum and in tap-water. The slides were incubated at 37.5° C and studied intermittently for two days. No immune precipitates formed about the larvae. The mortality rate of larvae in the experimental serum was not significantly different from that of the controls; all larvae were dead at the end of the study period, whereas Otto's controls lived for two weeks.

DISCUSSION

Hookworm species adapted to man as a final host penetrate beneath the superficial layers of the skin rather rapidly, sometimes without noticeable host reaction, then enter the blood stream and eventually migrate to the intestinal lumen. The depth to which "foreign" species penetrate the skin of man is apparently subject to some variation, depending as it would seem, on two separate factors, namely, the degree of adaptation of the hookworm species to man and the degree of resistance offered by the individual human host. Thus it has been known for some time that *A. braziliense,* the commonest cause of creeping eruption, wanders far through the cutaneous layers and survives for periods up to three months or longer (Kirby-Smith, Dove and White, 1926). *Uncinaria stenocephala,* the European dog hookworm, is somewhat less adapted to man, since Fülleborn (1927), while reporting lesions similar to those produced by *A. braziliense,* states that their duration is only three or four weeks. *Ancylostoma caninum* would seem usually to occupy the lowest position in the scale of invasiveness: Ellenbogen (1930) was unable to secure penetration of his skin by normal means; after blistering with cantharidin a few larvae did enter, but none produced a burrow and only one caused a small inflamed swelling at the point of entry. Other writers have described the typical *A. caninum* lesion as a papular or very short linear one that disappears within fourteen days.

The questions of allergy and sensitization in the rôle of these reactions are not fully answered by any means. Fülleborn (1930), who experienced distant wandering of a single *A. caninum* larva that entered the fingernail bed, said that he was sensitized as a result of previous *Strongyloides stercoralis* infection as well as a series of polyvalent helminth antigen injections. This result might seem to parallel the findings in the case of Subject B in this report. However, Heydon's case (1929), in whom wandering occurred also, was unresponsive to intracutaneous injection of *Ancylostoma* extract in spite of the fact that he had harbored an intestinal infection by *A. duodenale* for the previous two years.

Stumberg (1932) failed to mention eosinophiles in tissue sections through burrows of *A. caninum,* describing only the infiltrative findings common to mechanical trauma. Sandground (1939), Sandler (1938) and Taliaferro and Sarles (1937), however, all noted eosinophiles among other white cell accumulations about larvae that were being attacked or destroyed by the host. Eosinophiles—both in the blood and in solid tissues—may function as phagocytes, but this activity is a more character-

istic property of neutrophilic leucocytes. Therefore the presence of infiltrating eosinophiles in tissues invaded by helminths need not be regarded as a significantly added barrier to the worms' progression. It is, more probably, a chemotactic response, since the chief function of eosinophiles is believed to be to desensitize the host to parenteral foreign proteins (Ringoen, 1938), a concept entirely in accord with the association of eosinophilia with allergy. Allergic individuals, therefore, do not arrest the worms more effectively, or less so, than do non-allergic ones, and the explanation of the observed clinical variations must lie elsewhere.

A further note on the mechanism of host resistance to these worms may be made. *Ancylostoma braziliense* burrows are characteristically continuous with the points of entry of the larvae. *Ancylostoma caninum* burrows, as observed in Subject B and reported by Fülleborn and by Sandground, may be discontinuous, that is, the larvae penetrate to deeper tissues and later come to the surface at some distance from the points of entry (seven to eight inches distant in Subject B). It would seem therefore that *A. braziliense,* possessing invasive properties against a poorly resistant host, can wander rather freely through the skin despite the tissue's attempts to block it. *Ancylostoma caninum,* on the other hand, may be arrested at once, with resultant papular or negative reactions; or, if it can get through to deeper and less sensitive layers, it may then wander until it accidentally reaches the superficial skin once more. This would explain at once the two clinical types of reaction that have been described and the recurrent swellings experienced by Subject B over a period of many months.

SUMMARY

1. Two volunteers infected the skin of their right forearms with many filariform larvae of *Ancylostoma caninum.*

2. Subject A, without a past allergic history, experienced a short period of discomfort (under two weeks) with swelling and pruritus, followed by several months during which transient urticarial wheals appeared at or near the site of inoculation. He showed a maximum of 9% eosinophiles three months later.

3. Subject B, with a past history of multiple allergies, was hospitalized during most of the first month due to acute swelling of the entire forearm. A bacterial cellulitis was undoubtedly present, but in addition burrows appeared, extending discontinuously for seven or eight inches from the site of inoculation. During the succeeding several months he experienced occasional episodes of local or distant swelling, marked by burrow-like wheals. From a maximum of 26% he progressed into a gradually subsiding eosinophilia which fell below 10% after three and one-half months.

4. Neither subject showed eggs of *A. caninum* in the stools on the occasions these were examined; nor were there gastrointestinal symptoms suggestive of the presence of adult worms.

5. The host-reaction to various nematode larvae causing creeping eruption is discussed.

ACKNOWLEDGMENTS

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A NEW MITE, *LAELAPS APLODONTIAE*, FROM *APLODONTIA*[1]

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Several collections of mites from the mountain beaver, *Aplodontia rufa* (Rodentia: Aplodontidae), represent an undescribed species of the family *Laelaptidae*. This species is placed provisionally in the genus *Laelaps* although it does not have the characters of this genus in the restricted sense in which *Laelaps* is defined today. Particularly the ventral plate of the female differs from that of *Laelaps s.s.*, in having six pairs of spinelike setae instead of four and in its elongate shape.

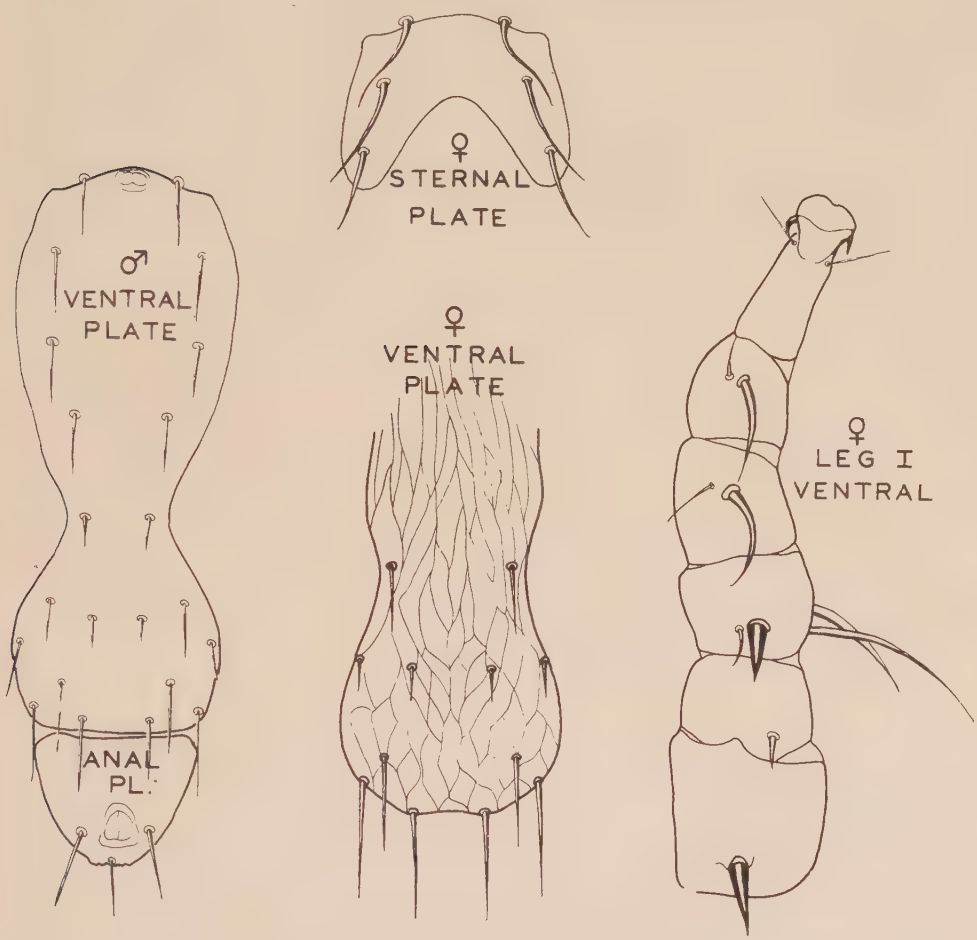

PLATE I. *Laelaps aplodontiae*, n. sp. Ventral and anal plates of male. Sternal and ventral plates, and leg I of female.

Laelaps aplodontiae, n. sp.

Female: Length 1 mm. The sternal plate, which is figured, is horseshoe-shaped with the anterior margin slightly convex and the posterior margin deeply concave. The sternal plate bears

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[1] From the Rocky Mountain Laboratory (Hamilton, Mont.), National Institute of Health.

3 pairs of setae of which the anterior pair is marginal. There is a fourth pair of ventral bristles posterior to the plate and opposite coxae III. The combined genito-ventral plate is figured. Its anterior margin is indistinct; it bears 4 rows of setae as follows, 2–4–2–4. The last row is marginal. The anal plate is separated by its width from the ventral plate. It is trapezoidal in shape and bears a pair of setae opposite the anal opening and a median posterior ventral seta. All legs are well armed with spines and bristles. Leg I is figured. It bears on the dorsal surface 2 long heavy bristles on article III and 4 spines on article IV. The tarsus has a pair of claws and a rounded pulvillus. There are ventral spines on all articles of leg I but are heaviest on articles I and III. The peritreme is long and slender, extending from the middle of coxa I to the posterior side of coxa IV. The spiracular opening is opposite the posterior margin of coxae III. Metapodal plates present but small. Chelicera of the pincer type with the immoveable finger slender and the moveable finger robust and slightly enlarged at the tip.

Male: Length 0.9 mm. Sternal, genital and ventral plate fused into one sclerite which is figured. Nine rows of setate on this sclerite arranged 2–2–2–2–2–4–2–2–4. The anterior row is marginal. The anal plate is contiguous with the ventral plate and is figured. Spines and bristles on legs are more robust than in the female. Article IV of leg I bears a specialized bristle which is broad at the base but bifurcates into a short, sharp prong and a long sinuous filament. Chelicerae flagelliform with long slender chitinous bases that extend posteriorly within the body to coxae IV.

The type series consists of the female holotype from *Aplodontia rufa,* Seattle, Washington, April 3, 1940, collected by W. A. Dalquest, and is from the collection of the United States National Museum. Allotype male, 2 paratype males, and 2 paratype females from *A. rufa,* Curry County, Oregon, May 26, 1935, from the collection of the Rocky Mountain Laboratory.

The type host, which is probably the normal host of this mite, is phylogenetically one of the most isolated of living rodents. It is the sole survivor of the superfamily APLODONTOIDEA, a family whose fossil history dates back to the upper Eocene (Wilson, 1937). Characteristic parasites of such an isolated mammal should be very distinctive. This is true of two monotypic genera of fleas, *Dolichopsyllus* Baker and *Trichopsylloides* Ewing, which are parasites of *Aplodontia.* It is also true of this new mite. No ANOPLURA or MALLOPHAGA have been reported from *Aplodontia.*

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THE CULTIVATION OF *TRICHOMONAS AUGUSTA* (PROTOZOA) FROM FROGS

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INTRODUCTION

According to the compilations of Walton (1941) and of Morgan (1944), *Trichomonas augusta* has been reported from 19 species in 7 genera of urodele amphibians, from 33 species in 9 genera of anurans, and from one species of lizard. *Trichomonas batrachorum* is also fairly common in amphibia, but has not been reported from so many kinds of hosts: 2 species in 2 genera of urodeles, 22 species in 7 genera of anurans, 2 lizards and one snake. From these lists one might assume that *T. augusta* is the more hardy species, and the more successful in adapting itself to different kinds of hosts. Yet, if one places a frog's rectal contents containing both species into some of the culture media commonly employed for trichomonad flagellates, *T. batrachorum* will grow in almost any of them while *T. augusta* usually does not grow at all or, if it does develop for a short time, it will soon be overwhelmed by the more rapidly growing *T. batrachorum*. This situation has been a challenging one and on several occasions I have assigned to graduate students the problem of trying to cultivate *T. augusta*. Until recently these efforts have not succeeded in maintaining this species in vitro for more than about 2 weeks. Unexpected success was attained in the winter of 1944–45.

METHODS

Cultures were maintained in ordinary test tubes with the usual cotton plugs, fluid media with added nutrients being employed. The basic fluid was either one-half strength Ringer's solution or the modified Ringer of Drbohlav (1925). For the most part the nutrients were gastric mucin and Bacto Loeffler's dried blood serum.

Specimens of *Rana pipiens* were the host animals. Original inoculations were from the recta of the frogs and contained the bacteria present therein. The usual precautions were taken to exclude organisms from the air. All cultures were maintained at room temperature.

Survival of the flagellates in individual culture tubes was prolonged by the addition of nutrient materials as needed and by adding distilled water to replace that lost by evaporation. In original cultures the mucin was dissolved by boiling, customarily in a concentration of 0.2%. Added nutrients were usually in the dry condition, an amount equal to about 0.2% being added at any one time. However, a stock of 0.4% gastric mucin in distilled water was kept on hand to use when both a nutrient and replacement water were to be added. For examination, a drop from near the top of the fluid, as well as one from the bottom, with sediment, was removed with a sterile pipette. Usually the flagellates were concentrated at the bottom, but often they were in the upper levels and occasionally were more numerous there than at the bottom. The following culture symbols will be used in the table and in the text: R^1 refers to regular Ringer's fluid, R^2 to Drbohlav's modification; L is used for the Loeffler's dried blood serum; M for gastric mucin and W for water; BH stands for

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Bacto dried beef heart, E–M for Bacto dried egg-meat medium, Li for Bacto liver powder, Ri for Bacto powdered rice, and Cer for cerophyl.

I am indebted to the Cerophyl Laboratories of Kansas City, Mo., for a sample of cerophyl and to the Wilson Laboratories, Chicago, Ill., for a supply of gastric mucin.

OBSERVATIONS

Having kept a number of strains of *Trichomonas batrachorum* under cultivation for some months, and desiring to obtain a new strain of this species, a frog was killed and opened on Dec. 9, 1944. When the rectum was removed it was apparently empty. It was cut in two and a little juice was squeezed from one part into R^2 on a slide. One specimen of *T. augusta* and a few of *Hexamitus* were observed. Assuming that a few individuals of *T. batrachorum* might be lurking in the rectal lumen, a piece was dropped into R^2 containing 0.2% gastric mucin (R^2M). Examination of the culture two days later revealed only *Hexamitus,* but 2 days after that a few individuals of *T. augusta* appeared. The numbers of *T. augusta* increased for several days and then began to decline. On Dec. 18, R^2 containing 0.1% of bile salts was added but the trichomonads did not increase. On Dec. 24 a piece of liver from another frog was dropped into the tube and in a few days the numbers had increased again. Then a decline set in. On Dec. 28, distilled water containing 0.4% of mucin was added and the numbers again increased. Water and mucin were added once more on Jan. 5, 1945, but on Jan. 9 the trichomonads appeared to be degenerating. Some dry beef heart was then added but *T. augusta* was not seen after that date. *Trimitus parvus,* which had appeared on the 5th day of culture persisted until the 66th day.

Trichomonas augusta had thus been shown to multiply in this culture which contained at first a piece of the rectum of the host, and later a piece of liver from another frog, and had persisted for 31 days. It seemed possible, therefore, that culture success might be attained if pieces of host tissue, especially pieces of rectum, were added to the media usually employed. However, a transplant into R^2M to which a piece of frog liver was added kept this species alive for only 12 days. Transplants into R^2M, R^2 with 0.03% peptone, R^2BH with 0.05% tryptone, and R^2M on Bacto-Entamoeba-medium slant did not keep *T. augusta* alive longer than 14 days, while *Trimitus* persisted for 184 days in the transplant containing originally a piece of frog liver.

This limited success in inducing *T. augusta* to multiply in a culture tube led to further attempts to cultivate it. But whenever *T. batrachorum* was present, it gradually increased in numbers till it apparently overwhelmed *T. augusta,* which did not persist longer than 24 days in any of the cultures containing both species. In the case of one frog, however, examined on Dec. 27, 1944, *T. batrachorum* did not appear in the cultures, and *T. augusta* was maintained as long as the experiment continued, until June 25, 1945.

Only 2 original cultures were made from this frog: A in R^2M (modified Ringer's with 0.2% gastric mucin), and B in R^2L, but the inoculum of each consisted of a piece of rectum with its contents. A number of years ago a student had found that *T. augusta* tolerated 0.4% NaCl better than stronger or weaker solutions. Consequently most of the subcultures were made in one-half strength Ringer's ($\frac{1}{2}R^1$). At the start of this series of cultures, it was deemed desirable to add new nutrient mate-

rials frequently and to vary them somewhat, hence gastric mucin and Loeffler's serum were commonly added alternately every 2 to 4 days. After the trichomonads had apparently become adjusted to cultural handling, various other methods were tried. Certain of the 26 cultures set up are shown in the table.

As seen in the table, original culture B, started in R^2L, was still positive when the cultures were abandoned, June 25, 1945, the flagellate having persisted in this tube for 180 days, while original culture A had become negative a few days previously. This is interesting, since the A culture had maintained a higher density of population through most of the period involved. The additions to A were limited to mucin, Loeffler's serum and water (see table) while B had also Bacto liver powder once and rice powder twice. Rice powder was first added when this culture was 72 days old and the next day many of the flagellates contained starch grains. Rice powder was

TABLE 1

Showing records of certain of the cultures into which *Trichomonas augusta* was inoculated. Original cultures are designated by a capital letter; subcultures by a number indicating the transplant generation followed by a small letter referring to each individual tube. Abbreviations: R^2, modified Ringer's; $\frac{1}{2}R^1$, one-half strength regular Ringer's; W, distilled water; L, Loeffler's dried blood serum; M, gastric mucin; BH, Bacto dried beef heart; E–M, Bacto dried egg-meat medium; Ri, Bacto powdered rice; Li, Bacto liver powder; Cer, Cerophyl.

Culture	Date	Original medium	Mucin added	Löffler's added	Other nutrients	Water added	Days of survival
A	Dec. 27, '44	R^2M	30	31	....	11	177
A/1a	Jan. 8, '45	$\frac{1}{2}R^1ML$	26	32	....	8	168*
A/1b	" 8, '45	$\frac{1}{2}R^1MBH$	3	..	BH6	2	54
A/1f	Feb. 14, '45	WML	2	1	....	..	9
A/1f^2	Mar. 3, '45	WML	12	12	....	3	77
A/1g	" 3, '45	WML	..	..	....	..	...
A/1g^2	" 9, '45	WML	1	..	....	..	8
A/1g^3	" 27, '45	WML	13	14	....	2	83
A/1i	" 3, '45	$\frac{1}{2}R^1L$	...	6	....	..	52
A/2a	Jan. 19, '45	$\frac{1}{2}R^1MBH$	18	14	BH6,Ri6	8	157*
A/2b	" 20, '45	$\frac{1}{2}R^1ML$	25	25	Cer2	7	156*
A/3a	Feb. 1, '45	$\frac{1}{2}R^1ML$	26	23	Ri4	8	144*
A/4a	Mar. 1, '45	$\frac{1}{2}R^1M$	23	..	....	1	72
A/4b	" 5, '45	$\frac{1}{2}R^1L$	..	4	....	..	12
B	Dec. 27, '44	R^2L	28	26	Li1,Ri2	8	180*
B/1a	Jan. 26, '45	$\frac{1}{2}R^1ML$	26	21	....	5	148
B/1b	Mar. 9, '45	$\frac{1}{2}R^1MLRi$	18	17	Ri3	3	108*
B/1c	" 9, '45	WE–M	18	2	Ri3,Cer2	3	101*
B/2b	Apr. 7, '45	$\frac{1}{2}R^1MRi$	18	..	Ri3	2	75
B/2c	" 7, '45	$\frac{1}{2}R^1LRi$	..	23	Ri5	1	77
B/3b	" 14, '45	$\frac{1}{2}R^1LRi$	..	21	Ri5	2	74*

* Still positive when abandoned, June 25, 1945.

then tried on other cultures and it was found that *T. augusta* will, in culture, ingest relatively large amounts of rice starch, until most of the cytoplasm is filled with granules.

Since the alternate addition of mucin and dried serum proved to be capable of maintaining this species, a test of the capacity of each substance separately was made. Culture A/1i was started with $\frac{1}{2}R^1L$ and the dry serum added every few days. The numbers gradually declined but persisted for 52 days. Culture A/4b, treated the same way, remained positive only 12 days. Culture A/2c was started with $\frac{1}{2}R^1ML$. Dry serum was added once when it was 8 days old, but all subsequent additions were of mucin. This culture remained positive for 104 days. Later these two nutritive substances were tried separately with starch. B/2b, with mucin and starch, remained positive for 75 days, M having been added 18 times and rice powder 3 times. B/2c, containing L and starch at the beginning, had L added 23 times and rice powder 5 times; it remained positive for 77 days. B/3b, a transplant of B/2c, started with

serum and rice powder, remained positive until abandoned on June 25, lasting 74 days. Rice powder was added 5 times and serum 21 times.

Bacto beef heart served fairly well as a nutritive material. Culture A/1b, started in $\frac{1}{2}R^1MBH$, had mucin added 3 times and beef heart 6 times. The trichomonads persisted 54 days. Culture A/2a, a transplant from A/1b, was initiated in $\frac{1}{2}R^1MBH$ and it remained positive for 157 days, until discontinued on June 25. But in this case beef heart was added only 6 times, while mucin was added 18 times and rice powder 6 times.

Some attempts were made to grow *T. augusta* in cultures made up in water. Culture A/1f set up in WML remained positive for 9 days. Eight days later it was reinoculated and remained positive for 77 days. Another attempt in WML was made (A/1g) but the flagellates did not survive. After 6 days this tube was reinoculated ($A/1g^2$), and the trichomonads survived for 8 days. Ten days later this same tube was again inoculated ($A/1g^3$) and the flagellates persisted for 83 days. Increase in osmotically active ingredients was probably a factor in improving the "conditioned" medium in these reinoculated tubes. Water and Bacto whole egg did not prove effective, but water and Bacto egg-meat (B/1c) did better, the culture remaining positive for 101 days, until abandoned; but during this period mucin was added 18 times, dry serum twice, rice powder 3 times, and cerophyl twice. Addition of cerophyl was not followed by any marked increase in numbers but addition of rice powder usually was. It was found advisable to add the rice powder in very small amounts and at relatively long intervals, since larger amounts appeared to be detrimental.

A trial was made of the technique of Kofoid and Swezy (1915) and of Rosenberg (1936): namely, that of sealing with vaseline the rectal contents from a frog in Ringer's fluid under a cover glass on a slide. After some days *T. augusta* gradually disappeared while *T. batrachorum* increased in numbers and eventually became the only species present. The latter persisted for about 4 months and then died out.

DISCUSSION

Existing literature provides a number of reports of the cultivation of *Trichomonas augusta* but they may not be reliable. Often when the contents of a frog or other amphibian are examined, they will be swarming with this species. *T. batrachorum* may also be present but in numbers too small to be noted. Usually, when such material is placed in culture media and examined some days later, large numbers of *T. batrachorum* may be present, while *T. augusta* has disappeared. If the examination is not too critical, the impression will be gained that the latter species has been successfully cultivated. Several times during the past 20 years in my laboratory, students have tried to cultivate *T. augusta* but always with disappointing results. The somewhat accidental success that is reported above may not be easily repeatable, but at least this one strain has been successfully cultured. At present the indications are that the following conditions are likely to result in the satisfactory culture of this species: (1) absence of *T. batrachorum,* (2) a weak saline solution (e.g., one-half strength Ringer's), (3) a piece of host rectum in the initial culture, (4) frequent addition of new nutrient materials which will act as substitutes for tissues (gastric mucin and Loeffler's dried serum together appear to satisfy this condition), (5) judicious additions of starch in the form of rice powder appear to be advantageous.

The initial partial success in cultivating *T. augusta* was perhaps associated with the presence of host tissues in the culture. Dried blood serum might be thought of as a good tissue substitute but it was not as effective as the combination with mucin. The latter apparently provided needed elements not available from the serum. One would suppose that beef heart and egg-meat media would be effective tissue substitutes but they did not seem to be as satisfactory as the serum-mucin combination.

The fact that *Trichomonas augusta* ingests starch so readily is interesting since it is a substance not likely to be present in the rectum of its host. Examination of tadpoles discloses that in these larval hosts, *T. batrachorum* is the more common species, while *T. augusta* is less common, and when found, is present in small numbers and is smaller in size. Yet tadpoles are supposed to be omnivorous and possibly more herbivorous than carnivorous. In adult frogs, on the other hand, which presumably are exclusively carnivorous, *Trichomonas augusta* is larger in size, has a high incidence and populations are often dense. *T. batrachorum* in adult hosts has a lower incidence and a lower density of population. Ordinarily *T. augusta,* when taken directly from the host does not contain solid food bodies, although its cytoplasm may be filled with small droplets of fluid (Kofoid and Swezy, 1915). *T. batrachorum,* by contrast, is an omnivorous feeder and commonly contains bacteria, *Blastocystis,* and other solid objects in its food vacuoles. Obviously, there is an intriguing problem of nutrition in relation to these two species that live together in amphibian hosts.

The maintenance of populations in cultures by adding nutrient materials and water suggests some interesting problems. One might suppose that very soon the accumulation of noxious waste products would inhibit or destroy the viability of the flagellates. The addition of mucin 30 times and dry serum 31 times within a period of 177 days, or an average of one addition every 3 days (culture A) might be expected to increase very greatly the concentration of osmotically active materials. Perhaps that is why this tube became negative. The necessity of discontinuing the cultures at the end of June, 1945, prevented the determination of the ultimate capacity of these flagellates to survive under the conditions provided.

SUMMARY

Trichomonas augusta is commonly found in great numbers in adult amphibian hosts and is often accompanied by *T. batrachorum* in much smaller numbers. When rectal contents of frogs containing these two species are placed in culture media usually *T. batrachorum* multiplies readily while *T. augusta* disappears within one or 2 weeks.

In my laboratory many attempts to cultivate *T. augusta* had failed. Partial success was attained when a piece of rectum containing this species was placed in modified Ringer's solution containing 0.2% gastric mucin.

Further trials showed that *T. augusta* may be cultivated under the following conditions: (1) absence of *T. batrachorum;* when this species is present it usually multiplies more rapidly than *T. augusta* and eventually overwhelms the latter; (2) use of a weak salt solution (e.g., one-half strength Ringer's); (3) addition of a piece of frog's rectum to the initial culture; (4) frequent addition (every 2 to 4 days) of some tissue substitute; alternate use of Loeffler's dried serum and gastric mucin was found to be successful; (5) judicious addition of starch in the form of powdered rice seems to be advantageous.

Under natural conditions, *T. augusta* appears to prosper better in adult amphibia while *T. batrachorum* seems to have the advantage in tadpoles. The latter species is omnivorous, feeding on bacteria and other organisms in the host's rectum. *T. augusta* seldom contains solid food bodies. Its avidity for starch, which it would not be expected to find in its carnivorous adult host, is somewhat surprising. Many problems of nutrition related to these two species of *Trichomonas* remain to be solved.

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PHYSIOLOGICAL OBSERVATIONS UPON A LARVAL *EUSTRONGYLIDES*. VIII. INFLUENCE OF RESPIRATORY POISONS UPON THE AEROBIC GASEOUS METABOLISM

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The larva of *Eustrongylides ignotus* which lives in cysts inside various fishes is a parasitic nematode that, contrary to many other endoparasites, leads normally a predominantly aerobic life. Two observations point in this direction. The first is that the blood-red color of the worms is due to haemoglobin dissolved in the body fluid (von Brand, 1937); the presence of such a concentration of this respiratory pigment could hardly be understood unless it were used to gather significant amounts of oxygen. The second observation represents an even more convincing argument. It was shown (von Brand, 1942) that a *Eustrongylides* exposed to an experimental anaerobic period accumulates an oxygen debt which is repaid over a period of several hours as soon as oxygen becomes again available. However, worms isolated from their cysts immediately prior to the determinations give very little evidence of having contracted there an oxygen debt; only during the first half hour is their oxygen consumption somewhat higher than the steady level reached after that time.

In view of this situation it seemed of interest to characterize the aerobic respiration of this organism further, and this was done by studying both the influence of respiratory inhibitors and stimulants. Some data were also gathered concerning the question whether anaerobic processes can supplement the failing aerobic respiration when the latter is inhibited by poisons. A study along these lines appeared the more desirable as so far very little is known about the nature of the aerobic respiration of parasitic nematodes. The only comparable investigation is that by Stannard, McCoy and Latchford (1938) who used the larvae of *Trichinella spiralis*.

MATERIAL AND METHODS

Only worms freshly isolated from *Fundulus heteroclitus* were used, and for every experiment new specimens were employed. As in the experiments reported upon previously in this series, from two to four, occasionally five, worms constituted an experimental lot. In most cases only the oxygen consumption was studied, but in some series the respiratory quotient was also determined. The respiration apparatus was the same Warburg apparatus used previously (von Brand, 1942, 1943); details of the experimental procedures (time of equilibration, rates of shaking, carbon dioxide determinations, etc) will be found in these papers. It will be sufficient to outline here the general procedure and to emphasize those points in which the present experiments differed from the earlier ones.

The worms were introduced immediately after isolation into the Warburg vessels containing 2 ccm of 0.85% NaCl solution, and their oxygen consumption was followed for two hours in order to establish their normal level of metabolism. As mentioned in the introduction, the oxygen consumption is above normal during the first half

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[1] The author is indebted to the Elizabeth Thompson Science Fund for a grant towards the purchase of the respiration apparatus used in this investigation.

hour. These values were consequently rejected when the initial rate of oxygen consumption was calculated.

At the end of the two hours the worms were transferred to new vessels containing the experimental solutions (in most cases 2 ccm of 0.85% NaCl solution plus various concentrations of poisons). This procedure was used in preference to adding the poisons through a sidearm in order to avoid having traces of the active substances distill over into the saline during the initial period. Many of the poisons used are not volatile, it is true, but it seemed preferable to use a uniform procedure in all cases. One drawback of the adopted method was that the vessels had to be reequilibrated to the temperature of the water bath before the first reading could be taken. Since this investigation is concerned with the maximal effect that a given substance exerts, the neglect of the first 20 minutes of exposure does not matter. The respiration within the experimental solutions was followed for two hours, in most instances. In some cases, however, in which in this time no noticeable effect had taken place, readings were continued for a total of 3 to 5 hours.

In the majority of those experiments that were of shorter duration, the worms were then transferred from the experimental solutions, after a thorough rinsing in 0.85% NaCl solution, to new vessels containing pure saline in order to test whether a recovery from the influence of the substances tested did take place. This period extended usually over 2 to 3 hours. At the end of these determinations the worms were removed from the Warburg vessels and kept over night in Erlenmeyer flasks containing saline, to give an opportunity of testing whether a permanent injury had taken place. In some series another determination of the oxygen consumption was carried out at that time (i.e., after 24 hours); it will be designated in the following sections as "second recovery period."

The temperature employed in all experiments was 41° C. It has been shown previously (von Brand, 1943) that the larvae are quite resistant against high temperatures in vitro, and it seemed desirable to have as high a metabolic rate as possible. It should be pointed out, furthermore, that the selected temperature is not unphysiological. The final hosts of the worms in question are herons, and, once the worms have been removed from the intermediate host (*Fundulus*), they correspond to juveniles that have reached the definitive hosts. Birds, however, are generally characterized by their high body temperature.

EXPERIMENTAL RESULTS

1. *The influence of inhibitors on the oxygen consumption.*—We shall consider in the following paragraphs the influence of poisons that are commonly considered as inhibitors irrespective of whether or not they actually produced such an effect in the present case. The following substances fall into this category: potassium cyanide, sodium azide, sodium arsenite, sodium pyrophosphate, iodo-acetic acid and ethyl urethane.

a. *Potassium cyanide.*—The respiration of the worms proved to be fairly sensitive against this poison. Concentrations between M/100 and M/3200 brought about practically the same degree of inhibition, while it was somewhat less pronounced in M/6400 (Fig. 1). The mean value of the initial period, for the former range of concentrations, was 109 ± 4.2 cmm O_2/gm/half hour; that of the KCN period 31 ± 1.5. The inhibition amounted, therefore, to 72%, while 28% of the respiration was cyanide resistant.

The worms themselves were not injured by the poison; they did not show any sign of damage, even after exposure to M/100 cyanide. In all cases the respiratory rate went back to a normal level. The time required for this recovery depended upon the cyanide concentration to which the worms had previously been exposed. In the stronger concentrations complete recovery did not occur during the 2 to 2½ hours of the first recovery period that followed immediately upon the exposure to the poison. But it was quite evident when the rate of oxygen consumption was determined after about 24 hours (second recovery period).

During recovery from the lower concentrations, distinct evidence of an excess oxygen consumption was found, i.e., the oxygen debt accumulated during the KCN period was at least partially repaid. This is clearest in the concentrations M/6400

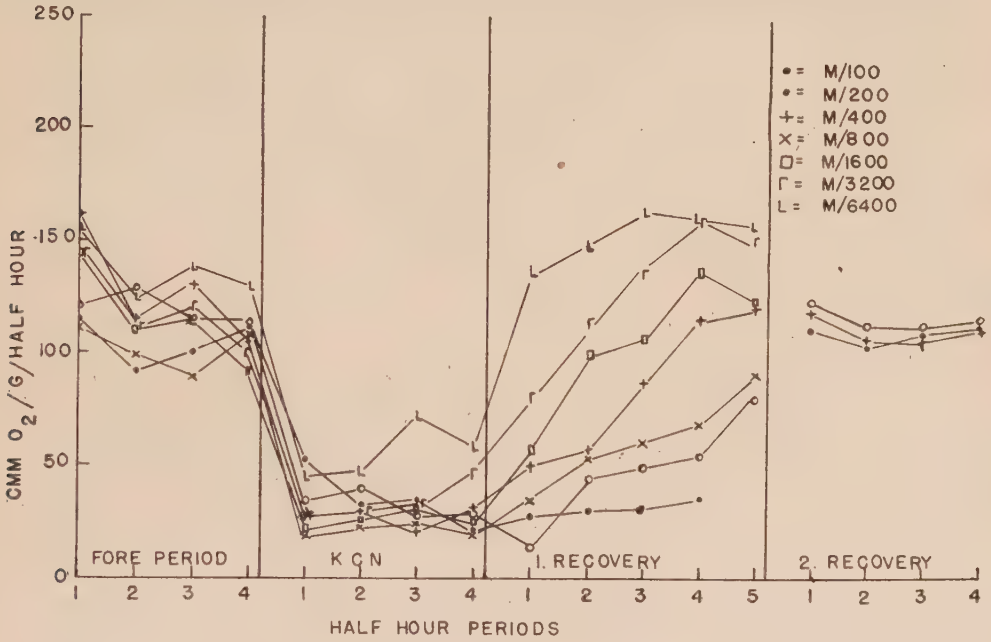

FIG. 1. Influence of various concentrations of potassium cyanide on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod, 1. and 2. recovery period: 0.85% NaCl; KCN period: specified concentrations of KCN dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Each point on the curves is the mean value of from 4 to 8 experiments.

and M/3200. The initial values for these series are 132 and 111 cmm O_2/gm/half hour respectively. The total amounts of oxygen missed during the 2 hours experimental period are 304 and 308 cmm. The figures for the total oxygen consumption during the first recovery period are 765 and 645 cmm. Had the oxygen consumption been at its normal level during this time, 660 cmm or 555 cmm, respectively, would have been consumed; i.e., 105 cmm were consumed in excess in the first case, 90 cmm in the second. This corresponds to roughly 30% of the incurred debt in both cases.

It is obvious that these figures are only approximations. On the one hand, the oxygen debt was actually somewhat larger than indicated since the equilibration period was not taken into account. On the other hand, the oxygen consumption had not quite dropped to the original level at the end of the period during which recovery was studied; in other words, one can expect that during the following hours still

more of the incurred debt would have been repaid. Whether a complete repayment takes place is doubtful; it should be remembered in this connection that it is still incomplete after enforced anaerobiosis due to lack of oxygen in the surroundings (von Brand, 1942). This is due to the fact that the worms are capable of excreting at least parts of the non-oxidized products of the anaerobic metabolism (von Brand, 1938), and it appears reasonable to assume that only those products that actually accumulate in the tissues give rise to excess aerobic oxidations.

Whether a comparable repayment occurs in the higher concentrations has not been investigated. If it does, it must have occurred during the interval between first and second recovery period.

b. *Sodium azide.*—Sodium azide was tested in concentrations varying between M/400 and M/8000; the pH in these experiments was adjusted to 5.0, since it is

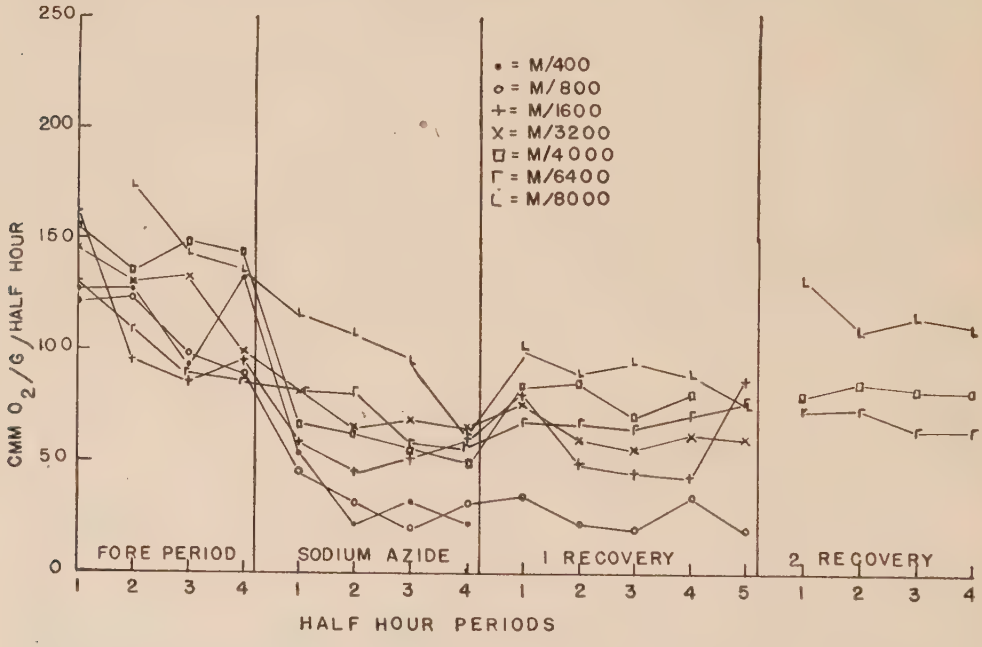

FIG. 2. Influence of various concentrations of sodium azide on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod, 1. and 2. recovery period: 0.85% NaCl; sodium azide period: specified concentrations of sodium azide dissolved in 0.85% NaCl. pH: 5; temperature: 41° C. Each point on the curves is the mean value of 4 experiments.

known that it exerts its most powerful influence in acid surroundings (Keilin, 1936). From a study of Fig. 2 it becomes apparent that the concentration of this poison is of much greater importance than in the case of cyanide. Maximal inhibition was observed only in M/400 and M/800 azide. The initial values of these two series were 117 and 103 cmm O_2/gm/half hour; those for the sodium azide periods were 32 cmm in both cases. The maximal inhibition obtainable with this substance was therefore about 69 to 73%, values very close to those found in the KCN series. In the lower concentrations the inhibition was gradually less pronounced.

The blocking of the oxygen consumption due to azide was more permanent than that brought about by cyanide. The data summarized in Fig. 2 show that full recovery occurred only in the lowest concentration used (M/8000) and then only after

24 hours. It should be emphasized, however, that this curtailment in oxygen consumption did not seem to harm the worms in any way. They appeared perfectly healthy when the experiments were terminated and were then just as active as before.

c. *Sodium arsenite, sodium pyrophosphate, iodo-acetic acid and ethyl-urethane.*—None of these substances had a statistically significant influence upon the oxygen consumption of *Eustrongylides*. It is, therefore, hardly necessary to present the data in as detailed a form as for the two preceding poisons. The pertinent figures have been summarized in Table 1.

TABLE 1

Oxygen consumption of larval *Eustrongylides ignotus* under the influence of sodium arsenite, iodo-acetic acid, ethyl urethane and sodium pyrophosphate. Media: Foreperiod and recovery period: 0.85% NaCl; experimental period: specified poison dissolved in specified concentration in 0.85% NaCl. pH: 7; temperature: 41° C. The values presented for the various concentrations are the means of 4 experiments in each case. The values presented under "average" are the mean figures derived from all experiments carried out with one of the specified poisons.

Foreperiod	Experimental period			Recovery period	
cmm O_2/gm/half hour	Concentration of poison	Time of exposure hours	cmm O_2/gm/half hour	Duration hours	cmm O_2/gm/half hour
		I. Sodium arsenite			
120	M/100	4	135	Not studied	
106	M/500	4	123	"	"
117	M/1000	4	125	"	"
Average: 114 ± 5.8			126 ± 7.1		
		II. Iodo-acetic acid			
127	M/800	2	119	2½	91
80	M/1000	2	99	2½	62
87	M/1600	2	95	2½	106
82	M/2000	2	84	2½	90
Average: 94 ± 4.9			99 ± 3.6		87 ± 5.2
		III. Ethyl urethane			
100	M/5	2	99	2½	98
111	M/10	2	97	2½	105
84	M/20	2	76	2½	77
110	M/40	2	88	2½	91
Average: 101 ± 4.9			90 ± 3.6		93 ± 4.2
		IV. Sodium pyrophosphate			
119	M/100	3	133	Not studied	
132	M/200	3	132	"	"
118	M/400	3	129	"	"
Average: 123 ± 5.0			131 ± 5.2		

The concentrations listed did not damage the worms. Sodium arsenite in a concentration of M/50 and M/500 iodo-acetic acid, however, killed a number of worms. No complete set of experiments could be performed with these concentrations and these data have consequently been omitted from Table 1. The lethal effect of the above two concentrations is nevertheless worthy of recording, since it indicated that at least sodium arsenite and iodo-acetic acid actually penetrate into the worms. Nematodes are covered by a strong cuticle and, if one gets negative results with a substance giving pronounced effects in other cases, the uncertainty remains as to whether it has passed into the tissues.

In so far as ethyl-urethane is concerned, a slight inhibition may have taken place. Although the differences are not statistically significant, one is tempted to assume that there has been an inhibition, because the value obtained during the experimental period was lower than those observed either during the initial or the recovery period.

2. *The influence of respiratory stimulants on the oxygen consumption.*—The following substances were tested: 2,4-dinitrophenol, para-phenylenediamine and methylene blue.

a. *Dinitrophenol.*—Dinitrophenol had a marked stimulatory effect on the oxygen consumption (Fig. 3). It was toxic in a concentration of M/250. The worms were still alive after the first recovery period, but, after being kept subsequently overnight in saline, a large percentage had died. An indication that the injury had taken place early can be deduced from the fact that the respiratory rate declined rather sharply during the first recovery period, in contrast to what was observed in the other concentrations. In the latter, the respiration remained high during the first recovery

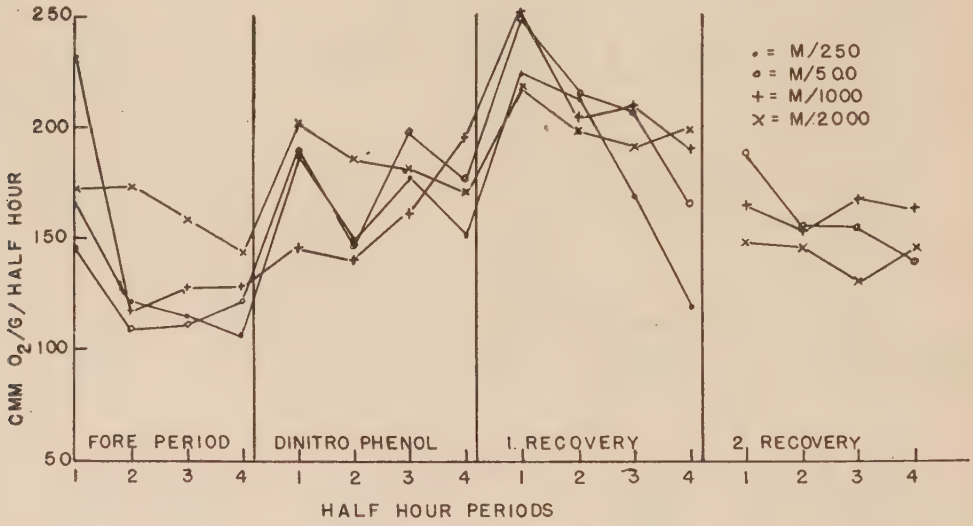

FIG. 3. Influence of various concentrations of 2,4-dinitrophenol on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod, 1. and 2. recovery period: 0.85% NaCl; dinitrophenol period: specified concentrations of dinitrophenol dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Each point on the curves is the mean value of 4 experiments.

period; as a matter of fact the level attained then was higher than either that of the initial or the dinitrophenol period. After 24 hours, however, the rate had dropped somewhat, but was still in excess of the normal values.

Although the variations between the different series were rather wide, no direct connection between the concentration and the rate of stimulation was apparent. In order to get a mean figure for the latter it appeared best, therefore, to average all the non-toxic concentrations (M/500 to M/2000). The following values in cmm O_2 per gram per half hour were obtained: Foreperiod: 132 ± 10.0; dinitrophenol period: 172 ± 8.1; first recovery period: 207 ± 13.2; second recovery period: 154 ± 7.6. The average maximal stimulation (difference between foreperiod and first recovery period) was therefore 75 cmm O_2/gm/half hour. This corresponds to about 57% of the normal respiration.

b. *Para-phenylenediamine.*—All the concentrations employed (M/100 to M/2000)

proved to be non-toxic. The results of these series are shown in Fig. 4. It is apparent that in all series a slight, but nevertheless distinct increase in the rate of oxygen consumption took place. There seems to be no connection between the degree of stimulation and the concentration of the active substance. It is therefore again justifiable to average the values of all series: initial oxygen consumption: 117 ± 5.4 cmm O_2/gm/half hour; para-phenylenediamine period: 140 ± 5.3; recovery period: 131 ± 4.9. The average excess oxygen consumption amounted consequently to 23 cmm per gram per half hour, or about 20% of the normal rate. The recovery value indicates that the influence of para-phenylenediamine is not as long lasting as that of dinitrophenol.

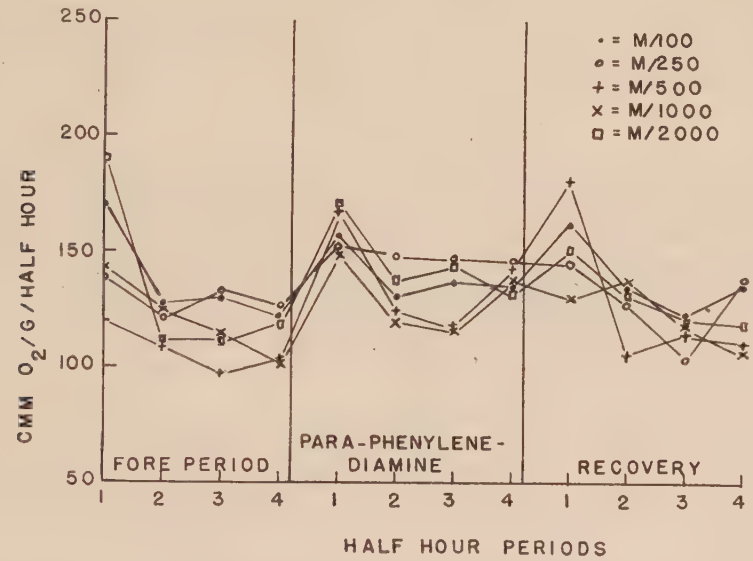

FIG. 4. Influence of various concentrations of para-phenylenediamine on the oxygen consumption of the larvae of *Eustrongylides ignotus*. Media: Foreperiod and recovery period: 0.85% NaCl; p-phenylenediamine period: specified concentrations of para-phenylenediamine dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Each point on the curves is the mean value of 4 experiments.

It should be noted that para-phenylenediamine is occasionally responsible for a much greater increase in oxygen consumption than indicated by the above figures. In a single experiment, in which the concentration was M/250 and which was not used in drawing Fig. 4 or calculating the average figures, the initial oxygen consumption was 140 cmm per gram per half hour. It rose during the experimental period to 352 cmm and fell during recovery to 185 cmm. The stimulation amounted in this case to about 160%. No satisfactory explanation can be offered for this aberrant behavior. The only difference between this and the other experiments was that the worms employed were somewhat smaller than usual (49 mgm per worm, as against 60 to 100 mgm in the majority of the other experiments).

c. *Methylene blue.*—Only one concentration was tested, M/400. The initial value was 107 cmm O_2/gm/half hour, during the experimental period (5 hours duration) the oxygen consumption averaged 118 cmm. The similarity of both figures certainly excludes any large-scale stimulation.

There was no clear indication that the dye had actually penetrated the worms.

In the next series, therefore, the initial oxygen consumption was first determined as usual, but the worms were then cut in half and immersed into M/400 methylene blue. As controls, similar experiments were carried out in which the respiratory rate of cut worms was determined in saline. The following values were obtained: Foreperiod, methylene blue experiments: 122 cmm O_2/gm/half hour; methylene blue period: 135 cmm. Foreperiod, saline experiments: 143 cmm; experimental period: 123 cmm. These values are again close, but nevertheless seem to support the idea that a slight stimulation may have been produced by the dye. It was certainly not much higher than 10% of the initial value.

3. *Influence of combination of poisons on the oxygen consumption.*—The series described in this section were designed to test in how far the increase in oxygen consumption that can be induced by various substances is cyanide sensitive. In the case of dinitrophenol and para-phenylenediamine the rate of oxygen consumption was

TABLE 2

Influence of cyanide on the oxygen consumption of larval *Eustrongylides ignotus* stimulated by para-phenylenediamine, dinitrophenol, methylene blue or potassium-chloride. Media: Foreperiod: 0.85% NaCl; experimental period: specified concentrations of para-phenylenediamine, dinitrophenol, methylene blue and their combinations with potassium cyanide in 0.85% NaCl. In the case of methylene blue the worms were kept prior to the first determination for 24 hours in M/400 methylene blue, dissolved in 0.85% NaCl; in that of potassium chloride, the worms were kept for a similar period in M/69 potassium chloride and the potassium cyanide used during the experimental period was in this instance dissolved in M/69 potassium chloride. pH: 7; temperature: 41° C. All the values presented are the means of 4 experiments. The duration of the experimental period was 2 hours in each case.

Foreperiod	Experimental period	
cmm O_2/gm/half hour	Active substances employed	cmm O_2/gm/half hour
123	M/250 p-phenylenediamine	145
111	" " + M/1000 KCN	33
93	M/500 dinitrophenol	181
92	" " + M/1000 KCN	35
Not determined	M/400 methylene blue	103
" "	" " " + M/400 KCN	28
" "	M/69 potassium chloride	147
" "	" " " + M/400 KCN	34

determined first for a period of 2 hours in the saline. This was followed in half of the experiments by a two-hour stay in the stimulating solution alone, while in the other half neutralized KCN was added to the stimulating solutions to bring the cyanide concentration to the strength specified in Table 2. Just as in the previous experiments, all these substances were prepared in a 0.85% NaCl solution.

A somewhat different procedure was used in the case of the potassium ion and methylene blue. It has been shown in a previous paper (von Brand, 1943) that potassium stimulates the oxygen consumption of *Eustrongylides* considerably when the worms are immersed into a KCl solution that is isotonic to 0.85% NaCl and that the increase is especially marked after a 24-hour stay in this solution. In the experiments reported here, therefore, the larvae were first kept for a day in a M/69 KCl solution and instead of an NaCl + KCN solution for the second half of the experiments a KCl + KCN solution was employed. Methylene blue was dissolved in 0.85% NaCl, but the animals prior to the determinations were kept for 24 hours in the dye solution in the desire to get as much methylene blue as possible into the worms.

The results of these experiments are summarized in Table 2. It is obvious that the level in oxygen consumption reached under the influence of cyanide was identical in all cases and closely corresponded to that induced by a pure cyanide solution (31 cmm, cf. a previous section). In other words, only the cyanide-sensitive part of the respiration was stimulated by dinitrophenol, para-phenylenediamine, the potassium ion and methylene blue, while the insensitive part remained entirely unaffected.

4. *Influence of poisons upon the carbon dioxide production and the respiratory quotient.*—In a number of experiments (Table 3), some of them identical with others mentioned in the preceding section, both carbon dioxide production and oxygen consumption were determined. The respiratory quotient during the initial period varied in the different series between 0.90 and 1.05. It has been shown (von Brand and Simpson, 1944) that only about 25% of the oxygen which the worms consume in sugar-free solutions at a comparable temperature can be attributed to the oxidation of stored carbohydrates. The relatively high values of the initial respiratory quotients must, in conformity with a previously expressed view (von Brand, 1942), be due to incomplete oxidations that proceed to some extent even at an oxygen tension of the atmospheric air.

The chief point of interest in the present experiments is whether, or to what extent, anaerobic processes are stimulated by the substances used. It is obvious that if the respiratory quotient rises significantly above 1.0 such processes must go on. The respiratory quotient alone, however, is not in itself a reliable index for the extent of the anaerobic processes, since its magnitude depends largely on the level of the oxygen consumption. Let us take two organisms with the respiratory quotient of 1.0 for the aerobic oxidations in both cases, but one consuming per hour 10 and the other 100 cmm oxygen. Let us further assume that both produce 50 cmm of carbon dioxide additionally from anaerobic processes. The overall respiratory quotient will be in the first case 6.0, but in the second only 1.5.

It was clear, therefore, that an attempt had to be made to calculate the actual carbon dioxide that originated on the one hand from the aerobic and on the other from the anaerobic metabolism. Consequently, the amounts of carbon dioxide originating aerobically during the experimental period were calculated under the assumption that they would have the same respiratory quotient as those of the initial period. This amount was then subtracted from the total carbon dioxide produced during the experimental period and the resulting figure was considered as "anaerobic carbon dioxide." The values thus obtained are probably not quite exact, since, as explained above, some incomplete oxidations are already present in the foreperiod, but there is no indication that their magnitude is of such an order as to vitiate the present calculation.

A survey of the figures presented in Table 3 show that para-phenylenediamine and iodo-acetic acid had no significant effect on the respiratory quotient. The very small amount of anaerobic carbon dioxide calculated for the latter poison is probably not real. Under the influence of potassium cyanide, on the other hand, a considerable evolution of anaerobic carbon dioxide took place, i.e., the worms were capable of supplementing the failing aerobic mechanisms through anaerobic processes. Of interest is also the influence of dinitrophenol. It stimulated not only the oxygen consumption, but it led also to a pronounced increase in aerobic fermentations. The increase in the respiratory quotient is relatively small, but this is due, as explained

TABLE 3

Influence of various poisons on the respiratory quotient of larval *Eustrongylides ignotus*. Media: Foreperiod: 0.85% NaCl; experimental period: specified poisons dissolved in 0.85% NaCl. pH: 7; temperature: 41° C. Length of foreperiod and of experimental period: 2 hours each. All the values presented are the means of 4 experiments.

Foreperiod			Experimental period					
cmm O_2/ gm/half hour	cmm CO_2/ gm/half hour	RQ.	Active substances employed	cmm O_2/ gm/half hour	cmm CO_2/ gm/half hour	RQ.	cmm CO_2 derived from aerobic processes	cmm CO_2 derived from anaerobic processes
96	89	0.92	M/1000 KCN	27	140	5.29	25	115
123	126	1.02	M/250 Para-phenylenediamine	145	142	0.98	148	0
92	86	0.93	M/500 Dinitrophenol	181	242	1.34	169	73
113	117	1.03	M/1000 Iodo-acetic acid	104	116	1.12	108	8
111	117	1.05	M/250 Para-phenylenediamine + M/1000 KCN	33	140	4.24	35	105
93	85	0.92	M/500 Dinitrophenol + M/1000 KCN	35	157	4.49	32	125
114	102	0.90	M/1000 Iodo-Acetic acid + M/1000 KCN	38	101	2.66	34	67

above, to the high rate of oxygen consumption. The carbon dioxide derived from anaerobic processes was actually about 2/3 of that produced under the influence of cyanide, that is when the aerobic oxidations are greatly restricted.

When para-phenylenediamine or dinitrophenol and cyanide were combined, the production of anaerobic carbon dioxide rose to the level reached under the influence of cyanide alone. The differences are clearly within the experimental errors.

A somewhat different picture was obtained when the combination iodo-acetic acid and cyanide was studied. The anaerobic carbon dioxide production was then only about 60% of that obtained in the cases mentioned above. This, of course, is due to the fact that iodo-acetic acid is a specific inhibitor of anaerobic processes. As explained in a previous section, the concentrations both of iodo-acetic acid and cyanide, as used in the present experiments, were non-toxic, if used alone. The combination of both substances, however, injured the worms rather rapidly. At the end of the 2-hour experimental period they appeared limp, although still able to move. But when kept, after the end of the determinations, overnight in pure saline, most worms had died. It follows that the elimination of a large part of the aerobic processes, for example through cyanide, does not interfere with the viability of the worms, probably because they can fill their energy needs vicariously through anaerobic processes. If, however, in addition the anaerobic energy production is partially interfered with, then the remaining mechanisms prove to be not powerful enough to keep the worms alive.

DISCUSSION

The respiration of the larva of *Eustrongylides ignotus* shows some similarities to that of the larvae of *Trichinella spiralis,* as described by Stannard, McCoy and Latchford (1938), but also distinct differences. Both organisms are tissue parasites and nematodes. They are both members of the same order (ENOPLIDA), but belong to different suborders (DIOCTOPHYMATA and TRICHINELLATA, respectively).

In both cases a marked and reversible sensitivity to cyanide was found. It was somewhat less pronounced in *E. ignotus,* since in the range M/100 to M/1000 the inhibition was 88 to 90% in *Trichinella* and about 70% in *Eustrongylides.* A noteworthy difference is that under the influence of cyanide no increase in anaerobic carbon dioxide production was observed in the former, while it was quite pronounced in the latter. *Trichinella,* therefore, shows no sign of a Pasteur reaction, *Eustrongylides* does.

Para-phenylenediamine stimulates the oxygen uptake of both organisms, while methylene blue either has no influence at all (*Trichinella*), or only a very slight one (*Eustrongylides*). The reaction of the two worms to arsenite is quite different. This poison in a concentration of M/100 inhibits the oxygen consumption of the former by 70 to 80%, while no inhibition whatever is seen in the latter. Whether a similar difference exists in respect to iodo-acetic acid remains to be seen; it may be noted that Stannard, McCoy and Latchford found a very pronounced inhibition of the oxygen consumption by means of iodo-acetamide.

A similarity between both worms, in which they differ from the alcoholic fermentation of yeast or muscle glycolysis, lies in the fact that iodo-acetic acid inhibits only slowly and incompletely the carbon dioxide production from anaerobic processes. This was found by Stannard, McCoy and Latchford in anaerobically conducted experiments, and confirmed in the present case where anaerobic processes induced by cyanide were studied.

Differences in the respiratory mechanisms of parasitic worms that are not very far apart in the taxonomic system, such as those described above, are of interest and should be an incentive for a comparative study of more forms. Aside from their theoretical interest, such investigations might in the future gain practical importance. It is well known that many worms show considerable differences in their reactions to anthelminthics. While so far, to the author's knowledge, a definite correlation between drug action and metabolic processes has not been established, it would appear quite possible that differences in the cellular enzymatic mechanisms lie at the root of these differences.

It has been shown that the oxygen consumption of *Eustrongylides* is inhibited by cyanide and azide but not by pyrophosphate. It is stimulated by para-phenylenediamine and the excess oxygen consumption induced by this latter substance is completely inhibited by cyanide. Exactly the same criteria apply to cytochrome oxidase (Keilin, 1929). It seems therefore very likely that a large part of the respiration is due to the Warburg-Keilin system.

There is very much less indication for the presence of aerobic dehydrogenases, since no marked inhibition could be achieved by means of urethane. In organisms operating largely on dehydrogenase systems, a considerable percentage of the oxygen consumption can be inhibited by these narcotics, in *Glaucoma,* for example, between 40 and 60% (Lwoff, 1934), in *Pelomyxa* between 36 and 65% (Pace and Belda, 1944).

No signs indicating a participation of glutathione or other substances containing –SH groups were found. Iodo-acetic acid and arsenite have been reported to interfere with the activity of these substances (Hopkins, Morgan and Lutwak-Mann, 1938; Lwoff, 1934), but they are quite without influence in the present case.

The enzymatic mechanism underlying the dinitrophenol stimulation is obscure. It is, however, remarkable that immersion into a solution of this substance leads not only to an increase in oxygen consumption, but at the same time to a marked appearance of anaerobic processes. A somewhat, but not exactly similar situation prevails in yeast. Dinitrophenol does not markedly change the oxygen uptake of *Saccharomyces cerevisiae,* but it stimulates to a considerable extent its aerobic fermentations (Pickett and Clifton, 1941).

SUMMARY

1. The oxygen uptake of the larval *Eustrongylides ignotus* is strongly inhibited by cyanide and azide, much less by urethane.

2. Pyrophosphate, iodo-acetic acid and arsenite do not interfere with its oxygen consumption.

3. The aerobic respiration is considerably stimulated by dinitrophenol, less by para-phenylenediamine and least by methylene blue.

4. Cyanide inhibits completely the increase in oxygen uptake due to dinitrophenol, para-phenylenediamine, methylene blue and the potassium ion.

5. Iodo-acetic acid and para-phenylenediamine have no influence upon the respiratory quotient. The latter rises, however, considerably under the influence of cyanide, indicating the presence of a Pasteur reaction.

6. Dinitrophenol stimulates not only the oxygen consumption, but also the aerobic fermentations.

7. Under the combined influence of dinitrophenol and cyanide or para-phenylene-

diamine and cyanide the same amounts of anaerobic carbon dioxide are produced as when cyanide alone is employed.

8. If a combination of iodo-acetic acid and cyanide is used, less anaerobic carbon dioxide is produced, but the action of iodo-acetic acid is less pronounced than in muscle glycolysis.

9. It is concluded that a large part of the respiration is due to an oxidase of the Warburg-Keilin type, that there is little indication for the presence of aerobic dehydrogenases. No signs of participation of substances with sulfhydryl groups were found.

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NOTES ON THE GAPEWORMS (NEMATODA: SYNGAMIDAE) OF GALLIFORM AND PASSERIFORM BIRDS IN NEW YORK STATE

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The importance of the gapeworm (*Syngamus trachea*) as a parasite of young gallinaceous birds is common knowledge. Since Klee (1903) first associated an outbreak of gapeworms in pheasants with the high incidence of the parasites in a nearby rookery, considerable interest in the possibility of cross-infection between the gapeworms of poultry and those of perching birds has been evidenced.

A number of British investigators have surveyed passerine populations for gapeworms (Lewis, 1925, 1926; Elton & Buckland, 1928; Morgan, 1931; Campbell, 1935), and artificial transmission of syngami from starlings, rooks, blackbirds (*Turdus merula*) to chickens has been accomplished (Leiper, 1926; Taylor, 1928; Rice, 1929; Clapham, 1934, 1935; Morgan & Clapham, 1934).

In this hemisphere Cram (1930) has reported *S. trachea* from some passeriforms in Alaska; Walker (1886), Manter & Pinto (1928), and Webster (1943) have recorded observations on gapeworms in robins; Cuvillier (1932) artificially transmitted gapeworms of pheasant origin to the house sparrow, and Ripple (1941) infected chickens with gapeworms of robin origin. No extensive surveys of passeriform birds for information on the occurrence of gapeworms have been made in this country to our knowledge.

Almost every morphological character proposed for the division of the genus into species (Leiper, 1913; Chapin, 1925) has been questioned (Chapin, 1925; Lewis, 1928). There has been no treatment of all the avian parasites of the genus since Cram's (1927) which could not consider the status of *S. merulae* Baylis, 1926, or the taxonomic problems indicated by the work of Lewis (1928). It is the purpose of this article to record observations on the gapeworms we have encountered in galliform and passeriform birds and to discuss the identity of the parasites.

MATERIALS

The hosts examined for gapeworms are listed in Table 1. The galliforms were submitted to the laboratory at various times between 1938 and 1944. All of the birds listed were from the wild. The passeriform birds were collected by various means during the period of March through December 1944. Some were shot, some were trapped, others were victims of predation or accidental trauma. The species collected represent a haphazard sample of the common forms which occur in the vicinity of several game farms and refuges in upper New York state.

Birds listed as juveniles were those in which the *bursa fabricii* was still present. No passeriforms were collected after December, so probably most of the juveniles were less than 6 months old. Grouse and pheasants, however, were taken during all months of the year and since the bursa persists into the winter in these birds, some of those recorded as juveniles were as old as 9 months.

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Specimens of *Syngamus* collected from the tracheae of the listed species were fixed in hot 70 per cent alcohol with 5 per cent glycerin, and were examined either in glycerin, after the evaporation of the alcohol, or in lactophenol. The number of worms available for morphological study is indicated in Table 2. Abundant *Syngamus* material collected from artificially propagated pheasants from the State Game Farms was also available.

OBSERVATIONS ON INCIDENCE

The incidence of gapeworms in the galliform and passeriform birds examined is indicated in Table 1. The generally accepted principle of greater susceptibility of younger animals to parasitism, is borne out by these data. It is obvious, however, that information from a survey of this type must be regarded as very general in nature, since the geographical origin of each infection in the migratory birds is usually uncertain and the incidence in the non-migratory game birds may depend greatly on the particular environment in which they are collected.

TABLE 1.—*Occurrence of* Syngamus *in wild birds in New York*

Hosts	Juveniles			Adults		
	Examined	Infected	Per cent	Examined	Infected	Per cent
Galliformes						
Ruffed Grouse (*Bonasa umbellus*)	301	3	1	303	0	0
Ringnecked Pheasant (*Phasianus colchicus*)	203	10	5	937	5	0.5
Passeriformes						
Eastern Crow (*Corvus b. brachyrhynchos*)	12	3	25	25	0	0
Eastern Robin (*Turdus m. migratorius*)	20	14	70	55	12	22
Eastern Meadowlark (*Sturnella m. magna*)	8	2	25	16	1	6
Bronzed Grackle (*Quiscalus versicolor*)	6	5	83	38	6	16

The following numbers of other birds have been examined for *Syngamus* and found negative: Hungarian partridge (*Perdix p. perdix*)—42; Eastern bluebird (*Sialia s. sialis*)—36; Starling (*Sturnus v. vulgaris*)—118; House sparrow (*Passer d. domesticus*)—50; Bobolink (*Dolichonyx oryzivorus*)—20; Eastern redwing (*Agelaius p. phoeniceus*)—39; Cowbird (*Molothrus a. ater*)—66; Slate-colored junco (*Junco h. hyemalis*)—22; Eastern tree sparrow (*Spizella a. arborea*)—20; Eastern song-sparrow (*Melospiza m. melodia*)—26.

Six of the 10 cases observed in juvenile pheasants occurred in a collection of 21 chicks taken in a limited area in the Lake Plains region. While the incidence there (6 out of 21, or 29 per cent) might be assumed from this collection, the figure of 5 per cent obtained from collection all over the state is likely to mean little in judging what infections might be expected in any one area. The 3 grouse which had gapeworms all occurred in the same covert in the southern Catskills and were, to the best of our judgment, all members of the same brood.

The absence of infections in adult crows and the incidence of 25 per cent in juvenile crows is in sharp contrast to the situation in the rook (*Corvus frugilegus*) in Britain, where Elton & Buckland found syngami in 4 out of 8 adults and 31 out of 33 juvenile rooks. It will be noted that no infections were observed in starlings although 118 (40 of which were juveniles) were examined. Lewis (1925, 1926) reported a gapeworm incidence of 35–37 per cent in this species in Britain and considered it to be an important distributor of gapeworms to poultry populations.

Since previous workers have not recorded the ages of the robins examined for

gapeworms it is difficult to compare our figures with theirs. Manter & Pinto (1928) reported gapes in 5 out of 7 examined; Ripple (1941) in 25 of 76 and Webster (1943) in 1 of 19. We have encountered no previous records of syngami in meadowlarks and bronzed grackles. Of the passeriform hosts from which Cram (1930) reported *S. trachea* in Alaska, we have had opportunity to examine only slate-colored juncos. No gapeworms were found in this species.

SIZE OF INFECTIONS AND PATHOLOGY

The numbers of worms encountered in infected birds is shown in Table 2. It is apparent that in most cases the juveniles harbored more parasites than the adults. There was no indication that either sex is more frequently or more heavily infected although the numbers we have examined may not be conclusive on this point. The

TABLE. 2.—*Numbers of worms encountered in* Syngamus *infections*

Species of bird	Age of bird	Number of pairs of worms
Ruffed Grouse	Juvenile	1 bird had 1 pr. 1 bird had 2 prs. 1 bird had 3 prs.
Ringnecked Pheasant	Juvenile	4 birds had 1 pr. each 1 bird had 2 prs. 2 birds had 3 prs. each 1 bird had 4 prs. 1 bird had 5 prs. 1 bird had 6½ prs.
	Adult	3 birds had 1 pr. each 2 birds had 2 prs. each
Eastern Crow	Juvenile	3 birds had 1 pr. each
Eastern Robin	Juvenile	1 bird had 1 pr. 2 birds had 2 prs. each 1 bird had 3 prs. 3 birds had 4 prs. each 1 bird had 5 prs. 2 birds had 6 prs. each 1 bird had 7 prs. 2 birds had 8 prs. each 1 bird had 45 prs.
	Adult	10 birds had 1 pr. each 1 bird had 2 prs. 1 bird had 3 prs.
Eastern Meadowlark	Juvenile	2 birds had 1 pr. each
	Adult	1 bird had 1 pr.
Bronzed Grackle	Juvenile	1 bird had lesions only 2 birds had 1 pr. each 2 birds had 2 prs. each
	Adult	3 birds had 1 pr. each 2 birds had 2 prs. each 1 bird had 3 prs.

birds which were regarded as adversely affected by their gapeworm infections are discussed in the following paragraphs.

In August, 1939, a grouse chick was found sick in the wild in the southern Catskills, was caught by hand and sent to the laboratory alive. Post-mortem examination revealed cachexia and the presence of 3 pairs of mature gapeworms, which occupied a large part of the trachea in this 179-gram bird. There was little food in the alimentary tract and no grit in the gizzard. When an investigator reached the locality in which the bird was found 12 days later and collected 2 more grouse, thought to be members of the same brood, it was found that both of the latter birds were also infected but neither showed any abnormal behavior.

In July, 1942, a pheasant chick, about 3 weeks old, was found dead on an area in Sussex County (Long Island) where population studies had indicated a high

brood mortality. Death of this bird was attributed to syngamosis; there was also a slight proventriculitis due to *Dispharynx* infection. Due to the paucity of the juvenile population only 2 other chicks were collected from the same area, 1 of which ($4\frac{1}{2}$ weeks old) harbored gapeworms and *Dispharynx,* the other ($1\frac{1}{2}$ week old) being negative. The $4\frac{1}{2}$-week-old chick had a severe proventriculitis. Intensive working of the area in September resulted in the collection of 10 pheasants, all adults. It is unfortunate that subsequent surveys have been impossible, since circumstantial evidence indicated such a high brood mortality in the presence of parasitism.

One of the robins examined was found dead, obviously as the result of severe parasitism. In addition to the complete blocking of the trachea by 45 pairs of gapeworms, the bird harbored numbers of *Dispharynx, Capillaria, Porrocaecum* and *Dilepis.* Another young robin, which was captured by a cat, showed severe tracheitis associated with the presence of 8 pairs of gapeworms, but in another bird with the same number of worms no gross pathology was observed.

MORPHOLOGY AND IDENTITY OF THE WORMS

All of the gapeworms examined during this investigation were identified as *Syngamus trachea* (Montagu, 1811) Chapin, 1925, except those which occurred in the robins, which we consider to be *Syngamus merulae* Baylis, 1926. The latter is readily distinguishable from *S. trachea* on the basis of the size and cuticularization of the buccal capsule.

The forms regarded as *S. trachea* exhibited many of the variations which Lewis (1928) observed. Certain variations occurred, however, in our specimens of *S. trachea* from the bronzed grackle, which were not noted by Lewis in his study. In most cases the dorsal ray in the gapeworms from the grackle was divided in two for its full length. Sometimes both branches were simple, sometimes one of them was bifurcate. It is difficult to say whether this arrangement most resembles the pattern in Lewis' specimens from rooks, starlings or blackbirds. The form of the tail of the female gapeworms from grackles was consistent and differed from any described by other authors. In all cases there was a constriction or neck near the end of the blunt tail, giving the posterior part of the female body an appearance somewhat like a Folin-Wu Blood Sugar Tube.

DISCUSSION

Leiper (1913) suggested the following characters be used for differential diagnosis in the genus *Syngamus:* (1) relative position of buccal capsules in paired specimens; (2) length of the oesophagus relative to that of the body; (3) size and armature of mouth capsule; (4) relation of axis of buccal capsule to that of body; (5) outline of optical section of chitinous wall of capsule; (6) size of spicules; (7) configuration of posterior end of body of female; (8) site of excretory pore.

Chapin (1925), however, questioned the use of most of these above characters and proposed the use of the configuration of the bursal rays as characters of specific value. The value of the ray characters, however, as well as those involving the shape of the posterior end of the body of the female, were made doubtful by the work of Lewis (1928) who indicated very considerable variations in most of the characters previously used to distinguish species. In a series of SYNGAMINAE from galliform

and passeriform birds from the British Isles he recognized only *S. trachea* (in chickens, turkeys, bantams, pheasants, starlings and rooks) and *S. merulae* Baylis, 1926 (in blackbirds). He pointed out that the blunt tails of *S. parvus* and *S. gracilis* did not distinguish them from *S. trachea,* but that *S. parvus* might be differentiated on the basis of the lateral bursal rays.

Cram (1927), following Chapin, presented a key to the avian species of *Syngamus,* using dorsal ray characters and the length of the spicules. Clapham (1940) remarked that "identification of the species of the genus *Syngamus* from corvid birds presents no unusual difficulties." In the light of Lewis' work, however, which heavily discounts the use of the dorsal ray as a taxonomic character, difficulties are encountered in the use of the above-mentioned key and in the evaluation of the validity of some of the species of *Syngamus* found in birds.

Probably the most readily usable character is the presence or absence of a large cuticularized rim about the anterior edge of the buccal capsule. It is this character, in the main, which has enabled the separation of *S. merulae* from *S. trachea* almost at a glance (Baylis, 1926; Lewis, 1928; Clapham, 1934; present paper). The absence of the cuticularized expansion on the buccal capsule is characteristic of *S. merulae* Baylis, 1928, and *S. microspiculum* Skrjabin, 1915 (from Skrjabin's drawings). These two species have similar dorsal rays (Skrjabin, 1915; Baylis, 1926; Lewis, 1928; Manter & Pinto, 1928; Ripple, 1941) usually divided for half their length, each branch being bifurcate, sometimes trifurcate.

Certain discrepancies exist in Skrjabin's descriptions of *S. microspiculum* which make the length of the spicules in that form a matter of speculation. Skrjabin's earlier paper (1915) reported the spicule length to be 0.15 mm and it was stated that no other species of *Syngamus* had such small spicules, although (as Chapin noted) the spicules in *S. trachea* had been indicated, by numerous authors prior to that time, to be about 60 μ. In 1916 Skrjabin gave the length of the spicules in *S. microspiculum* as 0.115 mm. Since in the latter paper he also gave 0.69 mm as the spicule size in *S. trachea* (apparently intending 0.069 mm), Manter and Pinto (1928) suggested that the mistake lay in the position of the decimal point and that 0.0115 (11.5 μ) was probably intended. By some calculation, inapparent to us, Clapham (1940) arrived at a figure of 49 μ for the spicules of Skrjabin's species.

That discrepancies in Skrjabin's figures are not always simply corrected by the moving of the decimal point has already been pointed out (Goble, 1941). Measurements on Skrjabin's drawing of *S. microspiculum* indicate that if the male body is 270 μ in diameter just in front of the bursa (as Skrjabin states) then the spicules are 88 to 90 μ long. This would appear to confirm the larger rather than the smaller measurements.

In describing *S. tenuispiculum* from the robin (*Turdus migratorius*) Manter and Pinto (1928) noted its similarity "to *S. merulae* Baylis (from *Turdus merula,* the blackbird) in size, buccal capsule, egg and spicules," but separated it on the basis of the dorsal ray. When Lewis' work appeared, they suggested (in a footnote, in press) that possibly their form would not be justified as a species separate from *S. trachea.* In 1941 Ripple conducted some experiments on transmission of the robin gapeworm to chicken and regarded *S. tenuispiculum* Manter and Pinto, 1928 as a synonym of *S. trachea.* Through the kindness of Dr. H. W. Manter we have been able to examine a pair of gapeworms from the group with which Ripple worked

and it is apparent that it is *S. merulae.* It is probable that all of his robin gapeworms were this species. We consider, therefore, that *S. tenuispiculum* is a synonym of *S. merulae,* not of *S. trachea.*

All species of avian syngami other than *S. merulae* and *S. microspiculum* appear to have heavily cuticularized expansions at the anterior edge of the buccal capsule. In this group are *S. trachea* (Montagu, 1811), *S. gracilis* Chapin, 1925 and *S. parvus* Chapin, 1925.

Chapin distinguished *S. gracilis* from *S. trachea* on the basis of the dorsal ray form, the inequality of the spicules, and the bent nature of the right spicule. Since the simple longitudinal splitting of the dorsal ray falls within the variations in ray pattern observed by Lewis and closely resembles the condition we have observed in gapeworms from bronzed grackles, this character is considered to be inadequate. Inequality of spicule length is quite common in the SYNGAMINAE, as is the bent condition of the right or longer spicule. Considering, therefore, that the characters proposed by Chapin are not qualitatively different from those typical of *S. trachea,* and that all recent collections of gapeworms from corvine birds both in Europe (Clapham, 1938, 1940) and North America (Beaudette, 1942; present paper) have been identified as *S. trachea,* it is regarded as probable that *S. gracilis* Chapin, 1925 is a synonym of *S. trachea* (Montagu, 1811).

The proper evaluation of the status of *S. parvus* Chapin, 1925 is difficult. Whether a spicule length of 49 μ can be used to distinguish a species from another species where the range is 53 to 82 μ is hard to say. In the light of what is known of variations in dorsal rays within this group, it is also difficult to judge the significance of the apparent stoutness and divergence of the lateral rays in *S. parvus,* in contrast to the slenderness and parallel arrangement in the other species of the genus. It is considered best to accord recognition to *S. parvus,* at least until material from either its type host, *Nucifraga caryocatactes,* or from other birds, becomes available for further study.

The following key includes the species of *Syngamus* occurring in galliform and passeriform birds. To our knowledge there is only one other avian species: described by El'perin (1938) from an owl (*Carine noctua*) in a Soviet publication which is unavailable to us.

I. Buccal capsule *without* heavily cuticularized anterior rim (II).
  A. Spicules longer than 90 μ ..... *S. microspiculum.*
  B. Spicules shorter than 90 μ ..... *S. merulae.*
II. Buccal capsule *with* heavily cuticularized anterior rim (I).
  A. Spicules longer than 50 μ. Lateral rays slender and parallel .... *S. trachea.*
  B. Spicules shorter than 50 μ. Lateral rays stout and divergent .... *S. parvus.*

SUMMARY

The incidence of gapeworms (genus *Syngamus*) in several species of galliform and passeriform birds in New York State is given. Records of *S. trachea* from meadowlarks and bronzed grackles are probably new. Data indicate significantly higher incidences in juveniles than in adults.

Observations were made on the size of infections and the pathological effects of the parasites on their hosts. The morphology and identity of the parasites were discussed. The form occurring in robins was considered to be *S. merulae* rather than *S. trachea;* the latter species occurred in all other species examined.

S. tenuispiculum Manter and Pinto, 1928 is considered to be a synonym of *S. merulae* Baylis, 1926. It is regarded as probable that *S. gracilis* Chapin, 1925 is a synonym of *S. trachea* (Montagu, 1811). A key to the species of *Syngamus* in galliform and passeriform birds is given.

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TWO NEW SPECIES OF ACARISCUS: *A. PLUVIUS* AND *A. ANOUS* (ACARINIDA: TROMBICULIDAE)

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Two species of chiggers were found on sea birds collected at Guam. A plover and a noddy from Bougainville were infested with one of the species. The presence of chiggers on sea birds whose migrations are extensive may explain in part the wide distribution of certain trombiculids. The hosts of the species under discussion, plover, tattler, and noddy, are noted for long flights over open sea.

Both of these chiggers are typical of the genus *Acariscus* Ewing, 1943. The genera *Acariscus, Trombicula,* and *Eutrombicula* are closely related. *Acariscus* is more closely related to certain species of *Trombicula* than to *Eutrombicula.* However, since *Trombicula* has a three-pronged palpal claw, and *Acariscus* has a two-pronged claw, they are readily distinguishable. The line of demarcation between *Acariscus* and *Eutrombicula* is not so well defined. Both genera have the two-pronged palpal claw, but they are separated on the number of dorsal and ventral setae. *Eutrombicula* has 22 dorsal and 14 ventral setae. *Acariscus* has more than 22 dorsal setae and more than 14 ventral setae with at least two pairs posterior to the anus. The number of dorsal setae in some species is 22, while the number of ventral setae is more than 14. Womersley (1944), who has had more experience with Eastern chiggers than any other worker, does not accept *Acariscus* but includes it in his concept of *Trombicula.* The taxonomic system of the TROMBICULIDAE is still somewhat fluid even at the generic level. It is the author's opinion that additional genera will have to be raised, or sub-generic categories will have to be introduced so that relationships can be shown more exactly. It is for these reasons that the broad concept of *Trombicula* is not accepted and the present species are placed in *Acariscus.*

Acariscus pluvius n. sp.
(Fig. 1)

The first specimen of this species was found on a plover collected on Cape Torokina, Bougainville. Its name is a contraction of *Pluvialis,* the generic designation of its host. Later, additional specimens were taken from two species of noddies and a tattler. The species was not found on mammals or land birds although many of these were examined in areas adjacent to the beaches where the sea birds were taken. Only engorged or partially engorged specimens were collected.

Body (Fig. 1, A and B) : The average length of five specimens was 470 microns; the average width 326 microns. The length and width vary with the stage of engorgement and are more a measure of the amounts of food ingested than they are specific characters. However, these measurements are significant in unfed and completely engorged specimens. The larvae are red in color. The striae are moderately developed anteriorly, but are weaker at the posterior end.

Palps (Fig. 1, C and D) : Segment 1 has a branched seta originating in the anterior lateral region; it usually bears 6 to 8 barbs or branches. The single seta on segment 2 is stout and has about 12 branches. Segment 3 has a single seta that is nude or may have a single fine barb. The type specimen has a simple seta on the right and one with a single fine barb on the left. The dorsal, lateral, and ventral setae of segment 4 are all nude. Segment five has 7 barbed setae and

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[1] On military leave from Duke University.

[2] The opinions expressed are the author's and not necessarily those of the Navy Department.

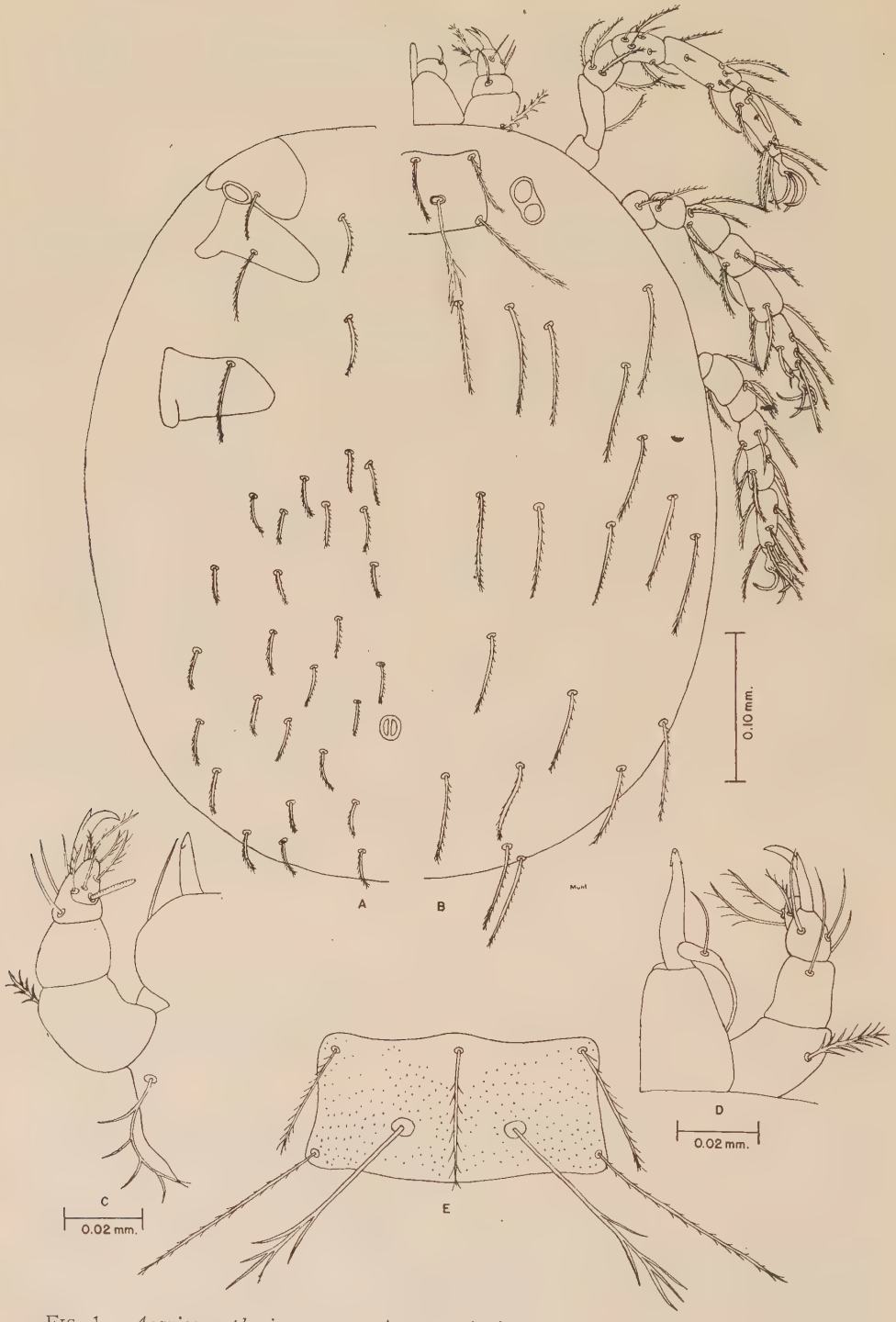

FIG. 1. *Acariscus pluvius* n. sp. A, ventral view; B, dorsal view; C, ventral view of capitulum; D, dorsal view of capitulum; E, scutum. ×500.

one long sensory seta. The sensory seta is striated and similar in other respects to those of other species. The bifurcate palpal claw is long and slender, and its median element is the stoutest.

Chelicerae (Fig. 1, C and D) : The basal segment is ornamented with a few small pits. It is about 37 microns long and 28 microns wide. The distal chitinous segment is strongly curved. The dorsal tooth is minute; the ventral tooth is small but recurved. The apex is sharp and points dorsally.

Legs (Fig. 1, A and B) : The legs are of the usual form. There is a single seta on each coxa. Tarsi I and II are provided with a sensory seta.

Scutum (Fig. 1, E) : The scutum is rectanguar in shape. It is ornamented by pitting except in the area of the anterior-median seta. The setae are provided with numerous short barbs throughout their entire length. The pseudostigmata are simple pits. The pseudostigmatic organs that arise from them are filiform and provided with branches along the distal two-fifths. The average standard measurements (Wharton, 1945) in microns of five specimens follow: AW—68, PW—76, SB—28, ASB—27, PSB—18, AP—25, AM—39, AL—37, PL—65, S—59, DS—65.

Setae: Both dorsal and ventral setae are similar to the setae on the scutum. The sternal setae, however, have slightly longer barbs than the others. The arrangement of the setae in this species is not constant. The most usual dorsal formula is 2–10–10–6–6–2–2, however, the second row may have as many as 13 setae, while the fourth row may have as many as 8. During engorgement the setae shift so that formulae such as 2–10–10–6–5–4–2 or 2–13–9–7–4–2 are found. The ventral setae are even more variable in arrangement. They are not arranged in transverse rows but more nearly in longitudinal ones. Posterior to the 4 sternal setae are about 50 setae. The anus is situated slightly posterior to the center of the group.

Hosts: Pluvialis dominica, Anous tenuirostris, Anous stolidus, Heteroscelus incanus.

Localities: Cape Torokina, Empress Augusta Bay, Bougainville Island, Australian Mandate; Ypao Point, Guam, Mariana Islands.

Type: U. S. National Museum.

Paratypes: U. S. National Museum; South Australian Museum.

Diagnosis: Acariscus pluvius is more closely related to *Acariscus anous* than to any previously described species. Both species can be differentiated from their closest relative, *Acariscus gliricolens* (Hirst, 1915),[3] by their more numerous ventral setae.

Acariscus anous n. sp.
(Fig. 2)

Specimens of this species were found on sea birds from Guam. *Anous,* the generic name of the noddy, has been adopted for this mite. Unengorged as well as engorged specimens were taken from the birds.

Body (Fig. 2, A and B) : The average length of five specimens is 420 microns; the width 320 microns. The type specimen is practically unengorged and measures 290 microns in length by 240 microns in width. The largest specimen measured was 560 microns by 410 microns. The larvae are red. The striae are well-developed anteriorly but are only moderately developed posteriorly.

Palps (Fig. 2, C and D) : Segment 1 has a branched seta with about 6 barbs. The seta on segment 2 is stout with as many as 12 barbs. Segment 3 has a seta that bears one or two fine barbs. The dorsal lateral and ventral setae of segment 4 are nude. Segment 5 has seven branched setae and one extremely long sensory seta. The bifurcate palpal claw has a fine lateral and stout median element. The cheliceral shield bears a pair of nude setae.

Chelicerae (Fig. 2, C and D) : The basal segment is lightly ornamented with a few pits. It is 48 microns long, 35 microns wide. The distal chitinous segment has a fairly well-developed ventral tooth, a sharp apex, and a minute dorsal tooth. It is strongly curved and points dorsally.

Legs (Fig. 2, A and B) : There is a single branched seta on each coxa and a sensory seta on tarsi I and II.

Scutum (Fig. 2, E) : The scutum is rectangular in shape. The ornamentation consists of numerous small pits that are more or less evenly distributed except in the region of the anterior-median seta where they are lacking. The pits are more numerous in this species than in *Acariscus pluvius.* The scutal setae are provided with short barbs along their entire length. The pseudostigmata are simple pits from which the distally branched, filiform pseudostigmatic

[3] New combination *Acariscus gliricolens* = *Trombicula gliricolens,* Womersley 1943 = *Microthrombidium gliricolens* Hirst 1915.

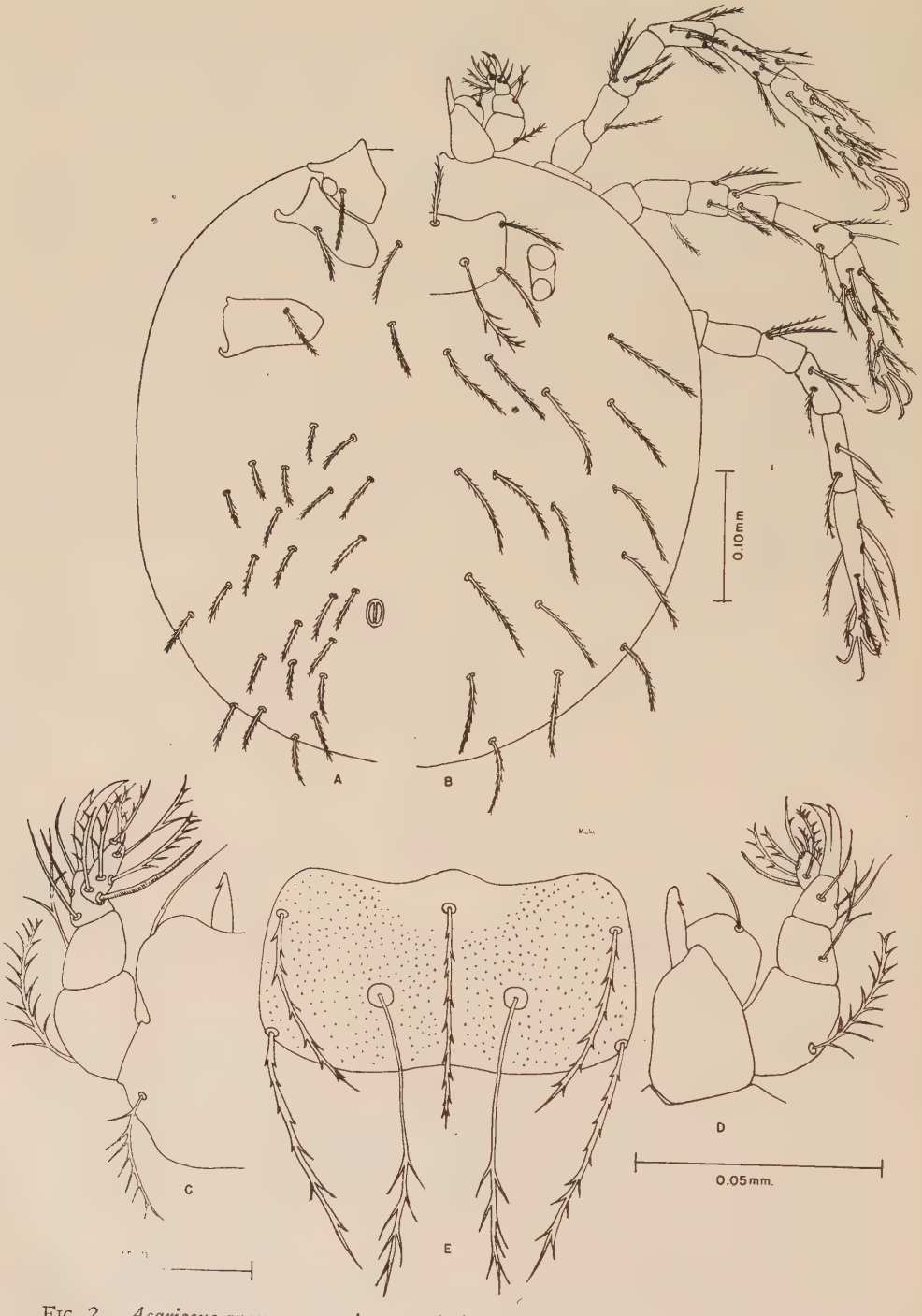

FIG. 2. *Acariscus anous* n. sp. A, ventral view; B, dorsal view; C, ventral view of capitulum; D, dorsal view of capitulum; E, scutum. ×500.

organs arise. The standard measurements (Wharton, 1945) were determined for five specimens. The means of these data follow: AW—93, PW—101, SB—37, ASB—32, PSB—19, AP—30, AM—32, AL—53, PL—75, S—73, DS—75.

Setae: The dorsal and ventral setae are similar to the setae on the scutum; however, the ventral setae are shorter than the dorsal. The dorsal formula is usually 2–8–2–8–6–4–2. Posterior to the two pairs of sternal setae are about 50 ventral setae arranged more or less in rows. The anus is situated posterior to the center of the group of setae.

Hosts: Anous stolidus, Heteroscelus incanus.

Locality: Ypao Point, Guam, Mariana Islands.

Type: U. S. National Museum.

Paratypes: U. S. National Museum; South Australian Museum.

Diagnosis: Acariscus anous can be distinguished from *Acariscus pluvius,* its nearest relative, on the following characteristics: the longer sensory seta on the palpal thumb, the larger more densely pitted scutum, and the different and more constant arrangement of the dorsal setae.

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COMPARISON BETWEEN IN VITRO AND IN VIVO GLYCOGEN UTILIZATION IN THE FOWL NEMATODE *ASCARIDIA GALLI*[1]

W. Malcolm Reid[2]

Glycogen appears to be the most readily available source of stored energy in intestinal parasites. Investigators agree that one-third or more of the dry weight of many intestinal parasites consists of glycogen and it is known to disappear at a rapid rate under starvation conditions. Most available information on glycogen metabolism has come from in vitro studies. Weinland (1901), Schulte (1917), von Brand (1937a and 1937b) and Ro (1939) studied the nematode, *Ascaris lumbricoides,* while Toryu (1935) used *Ascaris megalocephala.* Ortner-Schönbach (1913) used histological methods, while Alt and Tischer (1931), von Brand (1933) and Wardle (1937) studied the cestode, *Moniezia,* by chemical methods. Von Brand found that 11 per cent of the total glycogen store was utilized in six hours. If this rate were to continue, two or three days would be required to reduce the glycogen to a trace, since the glycogen content was 2.69 per cent of the wet weight of the parasite at the time of removal from the host. Markov (1939), using similar chemical analyses to study larval cestodes of fish, found that 94 per cent of the initial glycogen of the plerocercoid stage of *Diphyllobothrium latum* was utilized in 72 hours at 35° C in Ringer-Locke's solution. The glycogen metabolism of the trematode, *Fasciola hepatica,* was investigated by Weinland and von Brand (1926). Lapage (1937), Wardle (1937) and Baldwin (1943) have criticized the use of an unbalanced one per cent sodium chloride culture solution in many of these experiments. Furthermore, the discovery that worms survive better with aseptic techniques (Glaser and Stoll, 1938; von Brand and Simpson, 1942), makes it desirable to reevaluate the earlier in vitro work.

The effects of in vivo starvation upon the glycogen content of the fowl cestode, *Raillietina cesticillus,* and of the fowl nematode, *Ascaridia galli,* were reported by Reid (1942, 1945). In one experiment with *A. galli,* females consumed 71 per cent of the stored glycogen in 24 hours while in another experiment the reserve was depleted 75 per cent in 48 hours. With *R. cesticillus* the rate of glycogen utilization was even higher, 94 per cent of the stored glycogen being utilized in 24 hours. Thus far no chemical studies of in vivo starvation of either mammalian cestodes or nematodes have been made. However, Hager (1941) noted reduction in the egg production of *Hymenolepis diminuta* after starvation of the rat host while Chandler (1943) reported inability of tapeworms of the same species to establish themselves or to grow to maturity in hosts on a restricted carbohydrate diet.

Were it possible to compare simultaneously the glycogen consumption under in vivo and in vitro methods, the value of the earlier experiments would be more readily assessed. Investigations have seldom been carried out in such a way as to make this

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comparison possible. Von Brand (1938) studied the tissue parasite, *Eustrongylides,* in this manner, but concluded that the metabolism of the species resembled that of free-living nematodes rather than intestinal parasites. Before an investigation using both methods upon a typical intestinal form has been completed, comparison between the high in vivo glycogen utilization found in the avian parasites and the lower in vitro glycogen consumption of larger mammalian forms can have relatively little significance. A comparison between in vivo and in vitro glycogen utilization in the avian *A. galli* has been undertaken in the present study.

MATERIAL AND METHODS

The methods used in the care of chickens and in parasitizing of them, together with the methods for glycogen determinations, were described elsewhere (Reid, 1945). With *R. cesticillus,* cessation of feeding of the host during the night resulted in a reduction of the glycogen level from 7.13 per cent of the wet weight of the worm at 6 PM to 3.92 per cent at 6 AM. This reduction indicated that the available carbohydrate in the gut was exhausted soon after feeding had ceased. Since the fowls had an abundance of commercial mash, mixed for battery-reared chicks, before them at all times, they fed frequently but intermittently and thereby stored very little feed in their crops. The contents of the intestine diminished gradually, beginning two to three hours after removal of feed from the pens and the upper gut was almost entirely empty after the normal fast during the night. Accordingly, in the present experiment feed was removed at 6 PM and the fowls were killed 50 hours later at 8 PM. This resulted in approximately 48 hours of worm starvation although in some cases carbohydrate from the gut may have been available for a slightly longer period.

For the in vitro studies, one per cent salt solution as a medium for study of intestinal parasites has been criticized by Lapage (1937) and others. However, since it was desired to compare and assess the results obtained by other investigators, methods similar to those of von Brand (1937a) were used. Worms were washed in saline after removal and blotted dry on filter paper. In order later to have sufficient weight for the analytical methods, one female or two males were placed in individual weighed Erlenmeyer flasks which contained warm one per cent saline. The worms were weighed and placed in an incubator at $41.5° \pm 1°$ C, the temperature corresponding to the mid-afternoon temperature in the jejunum of fowls 57 days in age. Another criticism of the method commonly used with in vitro studies has been the maintenance of worms in an anaerobic environment. Slater (1925) concluded that *A. lumbricoides* became inactive and could not carry on normal activities when oxygen was excluded by the use of a hydrogen stream. However, since most other investigators have maintained their cultures under anaerobic conditions by this means, a hydrogen stream was passed through the flasks during the period of the present experiments. Although no attempt to bring about aseptic conditions was made, the saline was changed at the end of 24 hours. At the end of 48 hours worms were discarded from flasks which contained a nematode not responding to tactile stimulation while living worms were analyzed for glycogen.

EXPERIMENT

Chemical analyses were made on three groups of worms. Group I consisted of controls, group II of worms which had been starved within the host for 48 hours and

group III which had been starved in saline for 48 hours. Fowls, 59 days old, which had been parasitized with 300 embryonated eggs of *A. galli* 48 days before, were used as a source of mature worms. After killing some of the chickens the worms were recovered, separated by sexes and divided into two lots. One half of both the males and the females, used for group I controls, were placed immediately in potassium hydroxide for glycogen analysis. The other half, as group III, were weighed in warm saline and placed in the incubator for 48 hours. At the end of this period the parasites were killed for glycogen analysis. Group II worms were provided by depriving other fowls of feed for 50 hours and then removing and killing the nematodes under conditions similar to those of groups I and III.

The results of glycogen analyses from worms of the three groups are summarized in Table 1. In females, the glycogen store was reduced from 4.66 to 1.16 per cent after 48 hours of in vivo starvation while in vitro starvation resulted in reduction to 1.01 per cent. In males the glycogen store was reduced from 3.81 to 0.43 per cent under in vivo and 0.26 under in vitro conditions. Both males and females showed slightly greater glycogen utilization under in vitro than under in vivo conditions, the difference being 0.17 in males and 0.15 in females. Considering the difficulty in determining the onset of in vivo starvation and other variables inherent in the biological material used, these results show a remarkable likeness in glycogen utilization under the two conditions in the experiment.

TABLE 1.—*Glycogen utilization in* Ascaridia galli *during 48 hours of in vitro or in vivo starvation*

Treatment	Sex and number of worm samples analyzed	Mean sample percentage of glycogen of the worm wet weight	Percentage of glycogen store utilized in 48 hours
Group I Unstarved controls	10 F	4.66 ± 0.30* (3.04–5.78) †	
	9 M	3.81 ± 0.07 (3.55–4.09)	
Group II In vivo starvatin 48 hours	10 F	1.16 ± 0.10 (0.64–1.69)	75%
	10 M	0.43 ± 0.08 (0.12–0.82)	89%
Group III In vitro starvation 48 hours	8 F	1.01 ± 0.10 (0.72–1.44)	78%
	12 M	0.26 ± 0.03 (0.13–0.50)	93%

* Standard error. † Numbers in parentheses denote range.

DISCUSSION

The close similarity in glycogen utilization between parasites within the host and those in one per cent saline indicates that the results obtained by in vitro work of other investigators probably give a fair picture of this phase of metabolism within the host. Since a slightly greater amount of glycogen was used under in vitro than in vivo conditions, this conclusion seems more fully substantiated. It appears probable that neither the media used nor the anaerobic conditions introduced by using a hydrogen stream materially affected glycogen utilization in these earlier experiments. Furthermore, the short survival period of parasites noted by all investigators under these conditions was not due to an upset in the normal glycogen metabolism.

Comparisons between the mammalian *A. lumbricoides* and the avian *A. galli* are now possible. Von Brand (1937a) found that *A. lumbricoides* females consumed

45 per cent of the total glycogen in 48 hours while males used 49 per cent in the same period. Ro (1939), using the same species, obtained somewhat lower figures. Females used 38 per cent and males 23 per cent in 48 hours. In avian parasites the glycogen store was utilized more rapidly; 78 per cent in females and 93 per cent in males during the same period. Among the factors responsible for this more rapid metabolic rate may be mentioned the smaller size of *A. galli* which averaged 0.080 gram in females and 0.035 gram in males while in von Brand's experiments *A. lumbricoides* females weighed 4.14 grams and males 1.13 grams. Another contributing factor was the higher temperature with the avian parasite, 41.5° C, as compared with 37° in von Brand's experiments.

Ascaridia galli are so dependent upon a carbohydrate food source that most of them are expelled after 96 hours of host starvation (Reid 1945). It seems likely that *A. lumbricoides* could also be affected profoundly by host starvation, but the starvation period would need to be longer. Various commercial handling techniques of fowls may result in sufficient starvation to induce natural loss of parasites. It remains to be determined whether or not this is the case with mammalian forms. However, it appears probable that the differences in the normal glycogen content of *A. lumbricoides* found by various investigators in different countries as noted by von Brand (1937a) could be accounted for by differences in treatment of the host before slaughtering.

SUMMARY

1. Both male and female *Ascaridia galli* utilized approximately the same amount of glycogen during 48 hours of in vivo starvation as under anaerobic in vitro starvation in one per cent sodium chloride. Females consumed 75 per cent of their total glycogen store when starved within the host as compared to 78 per cent when removed and kept in the incubator. Males consumed 89 per cent when starved in the host gut and 93 per cent in saline.

2. Since glycogen utilization under the two different conditions in these experiments was similar, it may be concluded that the in vitro technique for study of glycogen metabolism probably has reflected satisfactorily the conditions in vivo.

3. Comparison of the glycogen metabolism in the avian *A. galli* in the present experiments with the mammalian *Ascaris lumbricoides* studied by other investigators indicated that the smaller parasite found in the avian host apparently utilized the glycogen reserve much more rapidly than did *A. lumbricoides*.

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DERMADENA LACTOPHRYSI N. GEN., N. SP. (TREMATODA: LEPOCREADIIDAE) AND CONSIDERATION OF THE RELATED GENUS *PSEUDOCREADIUM**

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Among trematodes collected from marine fishes at the Biological Laboratory of the Carnegie Institution at Tortugas, Florida, in 1930–1932, is a distome from trunkfishes, remarkable because of conspicuous glands with pores opening on the ventral surface. These glands are similar to those of certain monostomes (NOTOCOTYLIDAE). The study of these specimens involved consideration of *Distomum lamelliforme* Linton, 1907, from *Balistes* in Bermuda. The type specimen of *D. lamelliforme* was loaned by the U. S. National Museum through the kindness of Dr. E. W. Price. Numerous specimens of this same trematode collected in Bermuda by the late Dr. F. D. Barker were made available through the kindness of the General Biological Supply House. It is evident that Linton included material of two species under the name of *D. lamelliforme*. The type material, from *Balistes,* belongs in the genus *Pseudocreadium* Layman, 1930, while the specimens from *Lactophrys* evidently agree with my material from that same host at Tortugas and are referred below to a new species and genus of the LEPOCREADIIDAE closely related to the genus *Pseudocreadium.*

Dermadena lactophrysi n. gen., n. sp.
(Figs. 1–8)

Synonym: Distomum lamelliforme Linton, 1907, in part.

Description: Body circular or subcircular in outline; with edges inrolling ventrally except at anterior end; provided with cuticular scales, especially anteriorly, which may be lost; sometimes wider than long; length 0.765 to 1.822 mm, width 0.735 to 1.917 mm. A specimen 0.690 mm long was immature, one 0.765 mm long contained two eggs. Pigment spots present on youngest specimens, indicating an oculate cercaria. Ventral surface of body with numerous pores located on more or less elevated papillae; each pore is the opening of a conspicuous, multicellular gland; leading to the pore is a definite duct surrounded by radially arranged, vacuolated cells enclosed in a fibrous wall; vitelline cells frequently invade the basal portions of glands so that clusters of vitelline cells indicate the location of most of the glands. Number of glands from about 20 to over 90, arranged roughly in concentric rings or rows; number of rings increasing with body size (Figs. 2–4); five glands in acetabular region, two at anterior border of acetabulum, three directly over the gonads. Oral sucker subterminal, spherical or subspherical, 0.073 to 0.170 mm in transverse diameter; acetabulum near middle of body in immature specimens but somewhat anterior to midbody in older specimens; slightly larger than oral sucker, 0.097 to 0.197 mm in transverse diameter; sucker ratio 1 : 1.12 to 1.3.[1] Prepharynx lacking, pharynx subspherical, sometimes wider than long, 0.059 to 0.085 mm long by 0.044 to 0.107 mm wide; short esophagus; ceca narrow, distant from edges of body, with short diverticula or outpocketings, curving around gonads, ends usually not far apart. Genital pore slightly to the left, at midpharynx level. Testes two, symmetrical, intercecal, separated by ovary, immediately posterior to acetabulum, smooth or irregularly crenulated. Cirrus sac elongate, clavate, extending from genital pore diagonally backward to pass slightly beyond the right anterior edge of

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[1] In descriptions of trematodes, the ratio of the oral sucker to acetabulum is often expressed as 2 : 3, 3 : 4, 4 : 5, etc. It is proposed here to express this ratio with the value of the oral sucker always taken as 1. The result will be actually an acetabular index which can be directly compared with that of other trematodes (for example, 1 : 0.88 and 1 : 2.16). Another advantage is the expression of exact range of variation in sucker ratios.

acetabulum. Cirrus sac contains a basal, spherical, seminal vesicle; a prostatic vesicle of two parts each with large cells, the anterior part with more conspicuous nuclei; and a large cirrus, wider posteriorly, armed with short, wide tubercles or papillae. External seminal vesicle present. Ovary deeply trilobed, immediately posterior to acetabulum, between testes. Uterus short, preovarian, with few eggs; seminal receptacle present to left of acetabulum near metraterm; metraterm well developed, muscular, extending along left side of cirrus sac but diverging slightly posteriorly, about same length as cirrus sac, not overlapping acetabulum. Vitelline follicles widely distributed in dorsal portion of body from level of intestinal bifurcation to near posterior end of body, filling most of the wide extracecal fields, contiguous posterior to testes but not anterior to acetabulum. Eggs thin-shelled, 60 to 68 μ long by 34 to 49 μ wide. Excretory pore dorsal, conspicuous, with radial cells, relatively far anterior, not far posterior to ovary; excretory vesicle appears to be Y-shaped but the arms connect with the short stem by a narrow constriction (Fig. 8) and probably represent enlarged collecting vessels.

Hosts: Lactophrys tricornis (Linn.), trunkfish; present in 7 of 25 hosts examined.
Lactophrys trigonis (Linn.), trunkfish; in 9 of 11 hosts examined.
Lactophrys triqueter (Linn.), trunkfish; type host, in 2 of 4 hosts examined.
Location: Intestine.
Locality: Dry Tortugas, Florida.
Type specimen: U. S. Nat. Mus. Helminthol. Coll., No. 36931.

Diagnosis of the genus Dermadena: Family LEPOCREADIIDAE. Characters of the genus *Pseudocreadium* Layman, 1930, together with the possession of conspicuous ventral glands. Body with evanescent spines, subcircular, flattened, with ventrally inrolling edges. Testes symmetrical, ovary intertesticular; cirrus sac clavate, with basal seminal vesicle, bipartite prostatic portion, and tuberculated cirrus; external seminal vesicle present; cirrus sac and metraterm not extending posterior to acetabulum; seminal receptacle large, anterior to ovary; excretory pore dorsal, not far posterior to ovary; excretory vesicle often appearing Y-shaped, the lateral arms, however, probably enlarged collecting tubes extending between ovary and testes. Type species: *Dermadena lactophrysi.*

The generic name is from *derma* = skin, and *adena* = gland, referring to the characteristic ventral glands. The species name is for the host.

Discussion.—This trematode is like species of *Pseudocreadium* Layman, 1930 (= *Hypocreadium* Ozaki, 1936; *Leptocreadium* Ozaki, 1936) except for the ventral glands. Several species of *Pseudocreadium* possess the same circular body with inrolled edges and the same details of cirrus sac and female organs. The ventral glands are so striking and unusual that the new genus is based on them.

The smallest specimen, about 0.5 mm in length and immature, possessed about 20 very distinct, prominent glands (Fig. 2). The number of glands increases with age (Figs. 2–4) until in one of the largest specimens over 90 could be counted. The outermost glands are smallest and least developed. Most of the glands are over the area occupied by the vitellaria; they are not irregularly scattered, as might appear at first glance, but are arranged in more or less regular rows or rings approximately parallel with the body outline. Large specimens possess five or six, more or less concentric rows, while the smallest had three. There is always a pair of glands at the right and left anterior edge of the acetabulum, a large gland over each testis, and one over the ovary. These five glands in the acetabular region develop early. Just median to the ceca is a ring of 10 or 11 glands. Single glands on each side of the excretory vesicle do not seem to be a part of a regular row unless they be considered to form with the three glands over the gonads a central, complete ring of five. In the 0.5 mm specimen the first extracecal row of glands was barely beginning to form; in a 0.75 mm specimen it had 16 glands; in a 1.376 mm specimen it had 19 glands while a second extracecal row had 22 and a third extracecal row approximately 24. Thus, the innermost (that is, more median) glands seem to be constant in number and position but the outer rows are somewhat irregular, varying in number and with some glands not exactly in alignment. It is clear that additional outer rows of glands develop, and probably a few glands may be added to the extracecal rows anteriorly.

The glands apparently may be protruded to varying degree. When protruded, the tip is narrowed and the entire elevation is nipple-shaped (Fig. 5). A duct leads about halfway into the gland. Distal cells of the gland radiate from the duct and are largely vacuolated, with small, rounded, deeply staining bodies, probably nuclei (Figs. 6–7). At the base of the gland often six to eight vitelline follicles are closely clustered together and more or less enclosed by the fibrous lining of the gland (Fig. 6). This lining seems to be derived from the fibrous stroma of the parenchyma but it becomes less distinct near the base of the gland. Between these vitelline follicles and the distal cells occur a few, large, thin-walled cells which appear like disintegrating vitelline cells. They are filled with finely granular, yellowish, non-staining particles. However, some ventral glands are well developed before the vitelline follicles are differentiated and even in mature specimens some glands show no connections with vitellaria; for example, the two glands at the anterior border of the acetabulum. The glands directly opposite the three gonads are among the first to develop and usually bulge out, hernia-like, from the ventral surface. In some specimens much of the testes and almost the entire ovary and uterus seem to have moved into these sac-like bulges of the ventral surface of the body. A similar invasion of ventral glands by the testes, ovary, vitellaria, and uterus has been noted in the case of a monostome, *Quinqueserialis quinqueserialis* (Barker and Laughlin, 1911) (= *Notocotyle quinqueserialis*), by Barker and Laughlin (1911).

The presence of such ventral glands is unusual among trematodes and, so far as I can determine, previously undescribed for distomes. Papilla-like and retractile glands are well known in the monostome family NOTOCOTYLIDAE. Although the NOTOCOTYLIDAE are very unlike *Dermadena* in lacking an acetabulum, a pharynx, and a seminal receptacle, there are some interesting similarities in addition to the rows of ventral glands. The body of notocotylids is often concave ventrally and scaled or spined; the excretory pore is dorsal and far forward; the Y-shaped excretory vesicle of notocotylids is at least simulated in *Dermadena;* in both, the testes are symmetrical; the ovary is intertesticular; the cirrus sac and metraterm are both well developed; an external seminal vesicle is present. In addition, notocotylids sometimes possess the short diverticula of the ceca and a tuberculated cirrus. Since there is no doubt regarding the relationship of *Dermadena* to *Pseudocreadium,* a genus commonly recognized as in the family LEPOCREADIIDAE, evidence points to relationship between the monostome family NOTOCOTYLIDAE and the distome family LEPOCREADIIDAE. The few life cycles known in these two families include development of oculate cercariae in rediae, although the cercaria of *Notocotylus* is a monostome cercaria and the cercaria of *Lepocreadium* is distome and trichocercous. No life cycle of the genus *Pseudocreadium* is known.

Pseudocreadium lamelliforme (Linton, 1907), n. comb.

In 1907, Linton named *Distomum lamelliforme* from *Balistes capriscus* Gmelin (= *Balistes carolinensis* Gmelin), the triggerfish, and from *Lactophrys tricornis* (Linn.) and *L. trigonus* (Linn.) trunkfishes, at Bermuda. It is clear from Linton's description and figures and from study of type and other specimens from *Balistes,* the type host, that *Distomum lamelliforme* belongs in the genus *Pseudocreadium,* and that the specimens from trunkfishes are *Dermadena lactophrysi.* Linton did not describe or figure the ventral glands of the latter but the arrangement and size of

the structures he termed vitellaria in the specimens from trunkfishes make it clear that these were actually groups of vitellaria at the bases of the glands. The following measurements of *P. lamelliforme* are from three specimens on the slide of type specimens and six specimens from the "turbot" (*Balistes,* probably *B. capriscus*) at Bermuda. Length 0.999 to 1.952 mm, width 0.931 to 2.133 mm; oral sucker 0.082 to 0.142 mm in diameter; acetabulum 0.122 to 0.210 mm in diameter; sucker ratio 1 : 1.23 to 1.62; eggs 63 to 68 by 34 to 44 μ.

P. lamelliforme is so similar to *P. scaphosomum* Manter, 1940, that they should perhaps be considered the same. Sizes and sucker ratios agree. Eggs of *P. lamelliforme* average larger, with both upper and lower limits larger, although there is overlapping. The only detail observed which seemed to be constantly different is the shape of the external seminal vesicle. In all 47 specimens of *P. lamelliforme,* the external seminal vesicle is straight and sac-like, whereas in all of 31 specimens of *S. scaphosomum* the vesicle is tubular and sinuous, curved at least once and commonly S-shaped. Like *P. scaphosomum, P. lamelliforme* has a bipartite prostatic vesicle and when retracted the cirrus is somewhat folded or curved within the sac. *P. balistes* Nagaty, 1942, seems to differ chiefly in its bipartite external seminal vesicle and somewhat larger eggs.

SPECIES OF *Pseudocreadium*

The following eight species have been named in the genus *Pseudocreadium: P. monocanthi* Layman, 1930 (synonym: *Leptocreadium skrjabini* Ozaki, 1936—pointed out by Yamaguti, 1938); *P. vitellosum* (Ozaki, 1936) Yamaguti, 1938; *P. symmetrorchis* (Ozaki, 1936) Manter, 1940; *P. patellare* (Yamaguti, 1938) Manter, 1940; *P. scaphosomum* Manter, 1940; *P. spinosum* Manter, 1940; *P. sohali* Nagaty, 1942; *P. balistes* Nagaty, 1942; *P. elongatum* Nagaty, 1942; *P. lamelliforme* (Linton, 1907). Certain of these species, notably *P. elongatum* and *P. vitellosum,* are so similar to *Lepocreadium* that generic distinction between *Lepocreadium* and *Pseudocreadium* becomes questionable. Although the type species of *Lepocreadium, L. album,* has tandem testes and unlobed ovary, the genus, probably correctly, now includes species with diagonal testes and with lobed ovary, and some with ovoid as well as elongated bodies. Probably the best character to separate the genera is the symmetrical position of the testes in *Pseudocreadium.* In the type species, *P. monocanthi,* the multilobed ovary is directly anterior to one testis, in all other species with symmetrical testes the ovary is more or less intertesticular. It is suggested that species with diagonal testes be transferred to *Lepocreadium,* becoming *Lepocreadium vitellosum* (Ozaki, 1936), n. comb.; *L. sohali* (Nagaty, 1942), n. comb.; and *L. elongatum* (Nagaty, 1942), n. comb. Most species of *Lepocreadium* possess a terminal or subterminal excretory pore, while in *Pseudocreadium* the pore is dorsal, conspicuous, and relatively far forward. *L. vitellosum,* however, has the *Pseudocreadium* type of pore. The two genera approach each other so closely that separation must, at present, be more or less arbitrary.

Manter (1940) considered *Hypocreadium* Ozaki, 1936, a synonym of *Pseudocreadium,* pointing out individual variability of spination, lobation of ovary, and posterior extent of uterus. This view is still held, although a possible distinction could be the preovarian seminal receptacle and the intertesticular ovary in *Hypocreadium.*

The excretory vesicle in *Pseudocreadium,* as in *Dermadena,* usually appears to be Y-shaped due to the inflation of the lateral tubes. Sections of *P. scaphosomum* show that, in that species at least, the lateral vessels open into the vesicle slightly posterior to its anterior end.

Pseudocreadium galapagoensis n. sp.

In a restudy of the confusing individual variation of specimens of *P. scaphosomum,* it was discovered that the author's collection from *Balistes verres* from the Galapagos Islands contained two species rather than one. Of 13 specimens, 9 are small-sized *P. scaphosomum* as reported; the other 4 specimens are a new species. Five specimens collected from the same host, *B. verres,* from Isabel Island, Mexico, are all *P. scaphosomum.*

Pseudocreadium galapagoensis n. sp.
(Fig. 9)

Description (based on 4 specimens): Body slightly longer than wide, edge slightly inrolled ventrally in posterior half; 0.825 to 1.107 mm long by 0.750 to 0.931 mm wide; smooth except for a few fine scales on edges of suckers; oral sucker 0.127 to 0.217 mm in transverse diameter; acetabulum 0.144 to 0.225 mm; sucker ratio 1 : 1 to 1.13. Prepharynx short; pharynx relatively large, 0.102 to 0.136 mm long by 0.090 to 0.136 mm wide; esophagus short; ceca bow anteriorly and outward, then extend almost straight back until they curve medianly posterior to the testes, with short lateral and median bulges in posterior half. Genital pore to the left, opposite or just median to left cecum, about halfway between suckers. Testes symmetrical, lobed, in posterior half of body. Cirrus sac extending from genital pore almost directly backward along left margin of acetabulum to midacetabular level or slightly beyond; containing a spherical seminal vesicle, prostatic vesicle with wide posterior and narrow, tubular, sinuous anterior, portions; and sinuous cirrus. External seminal vesicle small, tubular, sinuous. Ovary of irregular, variable shape; seminal receptacle large, elongated, directly to left of ovary or to the left and slightly anterior, not overlapping acetabulum; uterus preovarian, extending along the right side of the acetabulum to or slightly beyond anterior edge of the acetabulum, then across the body posterior to the acetabulum to join the metraterm to the left of the cirrus sac; metraterm as long as or slightly longer than the cirrus sac, its posterior tip narrowed, appendix-like. Vitelline follicles filling sides of body from oral sucker to posterior end, confluent dorsally between pharynx and acetabulum and posterior to testes, continuous across ceca dorsally but not ventrally. Eggs 60 to 65 by 33 to 34 μ. Excretory pore dorsal at level of posterior ends of testes; excretory vesicle traceable only to the ovary.

Host: Balistes verres (Gilbert and Starks).
Location: Intestine.
Locality: Charles Island, Galapagos.
Type specimen: U. S. Nat. Mus. Helminth. Coll., No. 36932.

Comparisons.—The most characteristic feature of this species is the fact that both cirrus sac and metraterm lie parallel and close together on the left side of the acetabulum. In all other species these two organs diverge from the genital pore toward opposite sides of the acetabulum. The anterior coil of the uterus on the right side of the acetabulum and the postacetabular seminal receptacle are other peculiar characters. The suckers are more nearly the same size and the pharynx is relatively larger than in other species. *P. galapagoensis* is like *P. spinosum* in the preacetabular continuity of the vitellaria.

SUMMARY

1. A new genus, *Dermadena,* with type species *D. lactophrysi,* is named from trunkfishes at Tortugas, Florida.

2. The presence in *Dermadena* of rows of conspicuous ventral glands suggests similar glands known in the monostome family NOTOCOTYLIDAE and a possible relationship of the LEPOCREADIIDAE and the NOTOCOTYLIDAE.

3. *Distomum lamelliforme* Linton, 1907, is referred to the genus *Pseudocreadium*.

4. *Pseudocreadium vitellosum* Ozaki, 1936; *P. sohali* Nagaty, 1942; and *P. elongatum* Nagaty, 1942, are considered to belong in the genus *Lepocreadium*.

5. *Pseudocreadium galapagoensis* n. sp. is named for four specimens occurring with *P. scaphosomum* Manter, 1940, in *Balistes verres* from the Galapagos Islands.

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Explanation of Plate

All figures were made with the aid of a camera lucida. The projected scales have the values indicated in mm.

Abbreviations

cir	cirrus	gp	genital pore
cs	cirrus sac	mt	metraterm
ep	excretory pore	ov	ovary
esv	external seminal vesicle	prv	prostatic vesicle
ex	excretory vesicle	sr	seminal receptacle
gl	ventral gland		

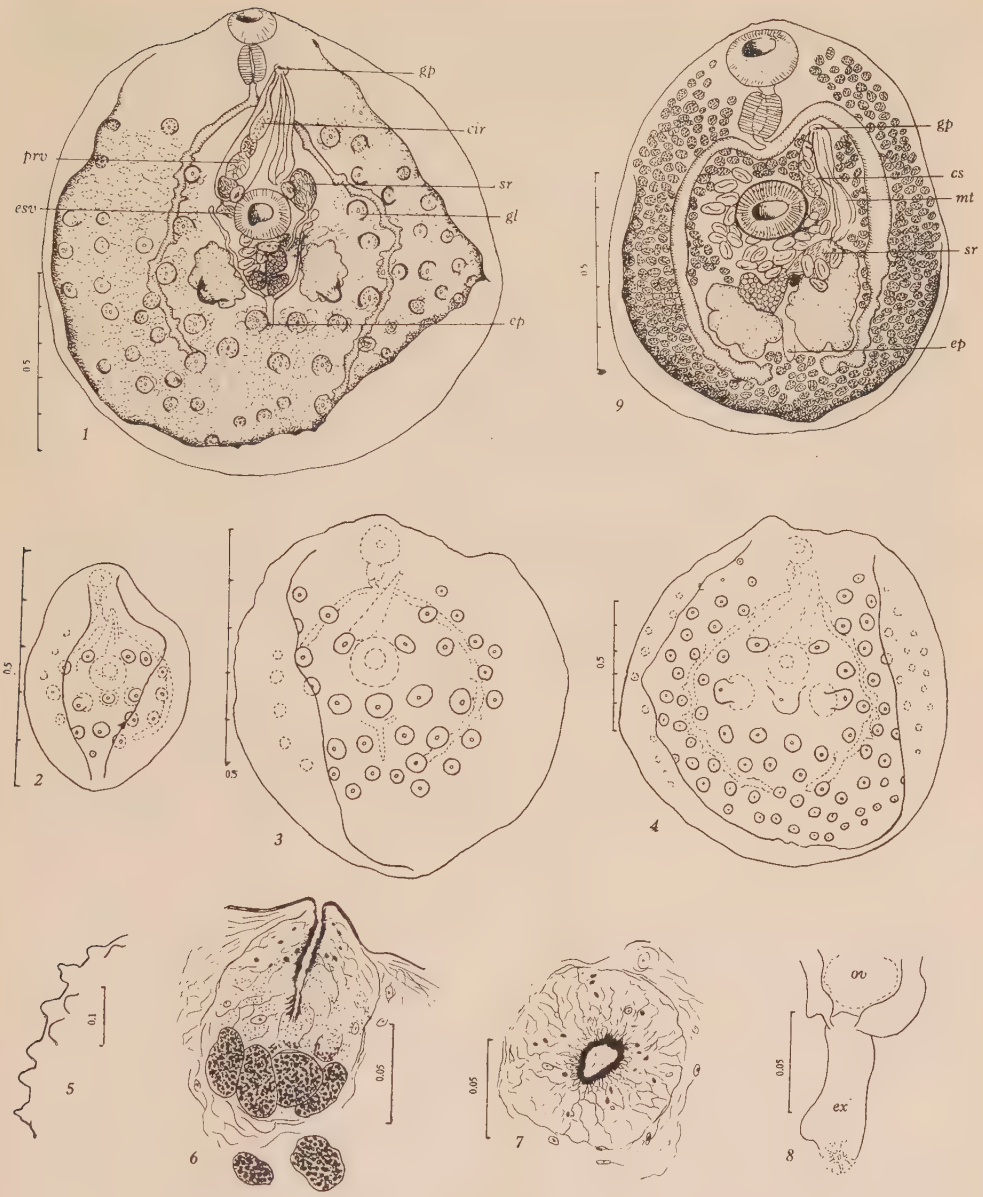

FIG. 1. *Dermadena lactophrysi.* Ventral view.

FIGS. 2–4. *D. lactophrysi.* Three specimens, 0.5, 0.75 and 1.376 mm in length, drawn to show number and position of ventral glands. Note change in scale in Fig. 4.

FIG. 5. Outline of portion of ventral body wall of *D. lactophrysi* showing protruded glands.

FIG. 6. Longitudinal section of a ventral gland of *D. lactophrysi* showing duct, vitelline follicles, and distal cells.

FIG. 7. Cross section through distal portion of a ventral gland of *D. lactophrysi.*

FIG. 8. Excretory vesicle of *D. lactophrysi.*

FIG. 9. *Pseudocreadium galapagoensis.* Ventral view.

REDESCRIPTION OF THE SPECIES OF *GYROCOTYLE* FROM THE RATFISH, *HYDROLAGUS COLLIEI* (LAY AND BENNET), WITH NOTES ON THE MORPHOLOGY AND TAXONOMY OF THE GENUS

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I. INTRODUCTION

In 1911 Edna E. Watson described *Gyrocotyle fimbriata* n. sp. from the ratfish, *Hydrolagus colliei,* collected on the California coast, and also reported the presence of a second species from the same host which she designated *"G. urna* (var.?)." Unfortunately Watson did not publish a detailed description of the second species, which she considered to be a variety of, if not actually identical with, the *G. urna* from *Chimaera monstrosa.*

Ward (1912) published a study of *Gyrocotyle* from the same host, but implied that there is only one species and that Watson had designated as separate species different stages in the contraction of the worm, with consequent differences in the amplitude and complexity of the lateral plications and of the folds of the rosette. Apparently, Ward considered *fimbriata* to be an invalid species, although he did not clearly say so.

Dollfus (1923) states that Ward was right in concluding that the two forms described by Watson belong to a single species (p. 236–237) and that *G. fimbriata* Watson is a synonym of *G. urna* Grube & Wagener (p. 238).

Wardle (1932) states that the *Gyrocotyle* from *Hydrolagus colliei* belongs to one species, *G. urna,* identical with the species from the Atlantic *Chimaera monstrosa.*

None of these authors, in disputing the presence of two species of *Gyrocotyle* in the ratfish, took into consideration any of the valid specific differences between them other than the differences in the complexity and amplitude of the plications of the lateral borders and of the rosette, which they regarded as inconstant and varying with the contortions of the worm. Ward and Wardle, at least, had both species at their disposal; it seems likely that Dollfus examined specimens of *G. fimbriata,* only.

In the past decade I have collected and examined hundreds of specimens of *Gyrocotyle* from ratfishes taken in Puget Sound and along the coast of Washington. The two forms described by Watson definitely exist, and there is no reason for not regarding them as distinct species. It is the purpose of this paper to describe both species with sufficient completeness for their subsequent recognition, and also to point out the inadequacies in the descriptions of several other species and alleged species of the genus in the hope that their precise taxonomic status will be defined by zoologists who have ready access to the hosts. Finally, certain disputed points in the interpretation of the morphology and development of the genus *Gyrocotyle* will be considered.

For details of the histology and cytology of the genus the reader is referred to the publications of Lönnberg (1891) and Watson (1911).

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II. MATERIAL AND METHODS

The ratfishes were usually obtained from commercial fishermen or from the collecting expeditions of students, and were examined from four to forty-eight hours after capture. Usually, at least twenty-four hours had elapsed from the time the fish were taken until the parasites could be removed. The intestine of the fish was removed and slit open in a dish of 0.7 per cent saline solution. The *Gryocotyle* were usually alive and in good condition, although the intestinal mucosa had sloughed off in the majority of the hosts at the time of examination. The worms were kept in the saline solution, or more rarely in sea water, until preservation. *Gyrocotyle* is notoriously difficult to fix in an extended and undistorted condition. The most satisfactory procedure was to narcotize them in the saline solution with menthol-chloral hydrate (Galigher, 1934). By the end of from six to eighteen hours some of the worms would be more or less normally relaxed, others would remain badly contracted. Relaxed specimens were dropped into 8% formalin in normal saline solution, or were fixed in saturated sublimate solution with 5 per cent acetic acid. Specimens which showed a tendency to curl were placed on a glass plate, straightened with camel's hair brushes, and flooded with the fixative. A few were fixed while strongly compressed between glass plates, but the resultant distortion nullified any advantages in the thinner specimens so obtained. Practically all stains adapted for whole mounts were tested on *Gyrocotyle*. Best results were obtained by staining with Galigher's (1934) modification of Harris's haematoxylin diluted with 9 parts of a saturated solution of ammonia alum. Carmines and cochineals have the disadvantage of leaving the spines unstained and practically invisible, and of not producing enough contrast between internal organs and the surrounding parenchyma. Alum haematoxylin usually stains the body spines, and sometimes the acetabular spines as well, and in addition produces more contrast between the organs of the reproductive system and the surrounding tissues.

III. ACKOWLEDGMENTS

The writer is especially indebted to Dr. Kelshaw Bonham, Mr. Frederick C. Cleaver and Mr. R. T. Smith of the State Department of Fisheries, to Captain Walt Hossfeld of the trawler "St. John" and to Captain Edwin Smith of the trawler "Garnet C." for donations of large numbers of ratfishes. Various other friends and students, too numerous to mention, have contributed occasional specimens. The photographs of Plate III were taken by Dr. Bonham.

IV. DEFINITION OF TERMS USED

In the descriptions which follow, the end with the acetabulum is considered anterior, the end with the funnel and rosette is considered posterior. The side bearing the small proximal pore of the funnel and the vaginal pore is designated dorsal, the side bearing the uterine pore and the male genital pore is ventral. Mature specimens are those bearing eggs in the uterus; immature specimens are those with adult characters, but without eggs in the uterus. Postlarval (juvenile) forms are those with reproductive organs incompletely developed and with incompletely formed plications in the rosette.

The funnel is the conical cavity extending from the posterior end of the body to the dorsal pore. The rosette is the elaborately plicated distal (posterior) border

of the funnel. The cephalic cone (Ward, 1912) is the region anterior to the genital notch and genital pores.

For convenience in description, the cuticular spines are considered to occur in the following groups: (1) acetabular spines, comprising two groups of large spines near the anterior end, to the right and left of the acetabulum; (2) anterolateral spines, forming a file of small spines along the lateral border between the acetabular group and the level of the anterior end of the uterine sac; (3) body spines, on the dorsal and ventral surfaces of the body. They are largest posteriorly and diminish in size gradually from behind anteriorly; (4) marginal spines, along the right and left margins of the body, posterior to the anterolateral spines. The last three categories of spines may not be sharply separated one from the others.

Unless otherwise stated, all data and discussions of a taxonomic character refer to mature specimens in a state of normal extension. Highly contracted, contorted or immature specimens were not used for measurements or comparisons.

With the partial exception of the inconspicuous and rather variable spines, *Gyrocotyle* has no structure of constant dimensions or pattern which can be used for specific identification. Since the animal is not only highly contractile and extensile, but also a gifted contortionist, and no two preserved specimens have precisely the same proportions or appearance, comparisons between species must be made to a large extent on the basis of the relative proportions, or degree of development, of conspicuous external and internal morphological features. Such a method is practicable in this case. If specimens of the two species of the same approximate size and state of extension are compared, homologous organs of the two species always show the same relative differences in size and position. Some structures of the two species present an absolute difference. For example, the length of the testicular areas in proportion to the length of the body, or the posterior limit of the vitelline glands, are constant differences without specific intergradations.

Several conspicuous morphological features distinguishing the two species are not amenable to convincing description. Such is the case with the size, number and amplitude of the undulations of the lateral borders of the body, and with the relative complexity of the rosette. Actually, the two species can be distinguished, almost invariably, on the basis of these two features alone. Contrary to the implications of several contributors to the literature on *Gyrocotyle* (Ward, 1912; Fuhrmann, 1930–31; Wardle, 1932), contraction or elongation does not convert the morphology of one into that of the other species. The species which, when elongated, has few and simple lateral undulations and a small, simple rosette, still has these features when contracted if compared with the other more complex species in the same stage of contraction.

V. TAXONOMY AND MORPHOLOGY

Genus *Gyrocotyle* Diesing, 1850 (Cestoda: Cestodaria)

Synonymy: Amphiptyches Grube and Wagener 1852; *Crobylophorus* Krøyer 1852–53.
Genotype: G. rugosa Diesing, 1850.

Diagnosis: Body of one segment, dorsoventrally flattened, in average extension about three and three-fourths times longer than wide and one-fifth to one-fourth as thick as broad, but highly extensile and contractile. At the anterior end a powerful muscular sucker (acetabulum); at the posterior end a funnel-shaped haptor with a small anterior dorsal pore and a wide posterior opening, the thin borders of which are more or less complexly folded (the rosette). Lateral borders of the body thinner than the median portion, simple, undulant or complexly ruffled. Cuticular spines present. Excretory pores paired, dorsal, in the anterior fourth of the body,

near the junction of the thicker median and thinner lateral regions. Vaginal pore dorsal, to right of the median line, in the anterior fourth of the worm. Ovary, shell gland and seminal receptacle in the third quarter of the body. Uterus courses anteriorly in spiral windings to open into a large saccate uterine pouch, the pore of which is ventral and median, in the anterior fourth of the body. Vitelline follicles extend almost the length of the body, are most numerous laterally and largely absent in the median reproductive area. Testicular follicles numerous, in right and left groups, in the anterior half of the body. Vasa efferentia empty into a coiled vas deferens which discharges into the ejaculatory duct through a valvelike papilla. Male genital pore ventral, to the right of the median line, in the anterior fourth of the body. Eggs thin-shelled, operculate. Larva ciliated, free-swimming, with a protrusible papilla bearing ten hooks at its posterior end. Life history incompletely known. Adults in spiral valve of Chimaerid fishes.

Gyrocotyle fimbriata Watson, 1911
(Fig. 1)

(For synonymy see Section X: Valid species of *Gyrocotyle*.)

Diagnosis: With the characters of the genus. Mature specimens 32 (13–63) mm in length. Acetabulum 1.86 (1.5–2.55) mm long; 0.90 (0.65–1.4) mm wide; slightly compressed dorsoventrally. Rosette large, its diameter 82 (60–112) per cent of greatest width of body, intricately folded, usually in contact with the posterior ruffles of the lateral margins. Undulant lateral borders extend from near the level of the genital pores to a short distance posterior to the dorsal pore of funnel, and are thrown into numerous, 31 (18–60), highly developed ruffles, the dorsoventral amplitude of which exceeds the maximum thickness of the body, and which are often replicate and secondarily undulant. Acetabular spines 25 to 50 in each group, not sharply set off from the anterolateral spines, the largest 175 (84–300) μ in length, from 6 to 13 per cent of the length of the acetabulum. Body spines encircle the posterior half of the funnel region anterior to the rosette, whence they extend forward dorsally to the level of the posterior part of the ovary, or very rarely to the posterior end of the uterine sac. Ventrally, the body spines rarely extend anteriorly to the funnel region. Posterior body spines 148 (75–250) μ long, sometimes larger than the acetabular spines. Scattered small spines occur along the margins of the lateral undulations in some specimens.

Excretory pores inconspicuous, dorsal, usually posterior to the level of the anterior end of the uterine sac, 3.4 (2.6–4.7) times the length of acetabulum from the anterior end. Testicular follicles in right and left groups, extending from near the middle of the acetabulum to, or beyond, the posterior end of the uterine sac. Left testicular area occupies 33 (24–45) per cent of total length of the worm. Vas deferens large, convoluted, conspicuous, discharging into the ejaculatory duct by a valve-like papilla surrounded by a conspicuous muscular bulb, both developed from the proximal end of the ejaculatory duct. Male genital pore ventral, slightly to right of median line, 11.5 (8.3–14.8) per cent of total length of the worm from the anterior end. Vaginal pore dorsal, about three-fourths of the distance from the median line to the lateral margin, and 10.7 (7.5–15) per cent of body length from the anterior end. Follicles of vitelline glands extend from about the level of the middle of the acetabulum to the level of the anterior border of the rosette.

Eggs spontaneously discharged from living worms 96.5 (90–107) μ×72 (58–78) μ, surrounded by a transparent jelly 5.5–9 μ thick; operculum present, with nearly invisible suture. Egg contents: an embryo in early stage of development, surrounded by vitelline cells.

Hosts: Hydrolagus colliei (Lay and Bennett) and *Chimaera monstrosa* L.

Gyrocotyle urna (Grube and Wagener, 1852)
(Fig. 2)

Synonymy: Amphiptyches urna Grube u. Wagener (in Wagener, 1852); *Gyrocotyle amphiptyches* Wagener, 1858; *Gyrocotyle nigrosetosa* Haswell, 1902.

(Following description based on specimens from *Hydrolagus colliei*.)

Diagnosis: With the characters of the genus. Mature specimens 36 (14–55.5) mm in length. Acetabulum 2.6 (1.7–2.75) mm long; 1.1 (0.93–1.45) mm wide, slightly compressed dorsoventrally. Diameter of rosette 45 (35–60) per cent of greatest body width, with few and simple plications. Thinner lateral margins of body with few, 15 (8–30), undulations which extend posteriorly from near the level of the genital pores to disappear between the level of the ovary and dorsal pore of the funnel; the undulations may be absent in small specimens. Acetabular spines compactly grouped, 17 to 25 on each side, the largest 200–800 μ long; from 9 to 27 per cent of the length of the acetabulum. Body spines numerous, extending from mid-region of funnel anteriorly to the region of the uterine sac on the dorsal side; ventrally, they extend anteriorly little beyond the level of the dorsal pore. Marginal spines may occur the entire length of the worm, but usually are large, numerous and conspicuous only posterior to the level of the ovary. Posterior dorsal spines large (150–360 μ) becoming gradually smaller anteriorly.

Excretory pores conspicuous, dorsal, usually anterior to the level of anterior end of uterine sac, 1.7 (1.3–2.5) times the length of acetabulum from the anterior end. Testicular follicles in right and left groups, extending from about the level of the posterior fourth of acetabulum to the level of the anterior border of the uterine sac, or rarely to the middle thereof. Left testicular field 11 (7–15) per cent of total length of worm. Vas deferens inconspicuous, feebly convoluted. Ejaculatory duct with papilla in its proximal end which is not surrounded by a conspicuous muscular bulb. Male sex pore ventral, slightly to the right of median line, 10.6 (7.8–14.7) per cent of total length of worm from the anterior end. Vaginal pore dorsal, usually about half way between the median line and lateral border, 9.4 (7–14.1) per cent of total body length from anterior end. Vitelline glands extend from the middle of acetabulum to a level anterior to the dorsal pore of funnel.

Spontaneously deposited eggs 94 (81–111) μ by 65.5 (55–72) μ, operculate, but with nearly invisible suture; entire egg surrounded by a transparent jelly 6–12 μ thick. At time of deposition, egg contains an embryo in early stage of development surrounded by vitelline cells.

Two varieties: Forma *magnispinosa,* with largest acetabular spines 420–800 μ long, always longer than body spines. Forma *parvispinosa,* with largest acetabular spines 220–360 μ long, usually smaller than largest body spines.

Hosts: Hydrolagus colliei (Lay and Bennet); *Chimaera monstrosa* L.; *Chimaera olgibyi* Waite; and doubtfully *Callorhynchus antarcticus* Lacépède.

Discussion of Specific Differences

1. *External appearance.*—The two species can usually be distinguished with the unaided eye by the following criteria: *G. fimbriata,* as compared with *G. urna,* is more robust, the lateral ruffles are more numerous and protruding, the rosette much larger and more intricate, the sides of the body more nearly parallel with less taper in the posterior fourth, the "cephalic cone" is slightly larger and more conspicuous, and the body spines with their papillae are not evident.

2. *Size and complexity of the rosette.*—In all specimens past the postlarval stage the rosette (the expanded, plicate border of the funnel) is enormously more complexly folded and crispate in *G. fimbriata* than in *G. urna.* In addition it is relatively nearly twice as wide, on the average, as in *G. urna,* although the mobility of the worms results in an occasional specimen of *fimbriata* presenting a contracted rosette of *urna*-like dimensions, but not of *urna*-like simplicity.

3. *The lateral ruffles.*—In *G. fimbriata* the lateral undulations are usually more numerous than in *G. urna,* always of greater dorsoventral amplitude, always more outstanding and distinctly separated from the median thickened part of the body, usually more flaring at their outer borders, and are more likely to possess secondary undulations or lobes. If one semicircular curve of the border be considered a ruffle, *G. fimbriata* will possess, on the average, twice as many ruffles as *G. urna.*

4. *The cuticular spines.*—In living specimens of both species the spines of all parts of the animal are usually colorless and glassy, although occasionally a light yellow. They are rather soft, and shrink more or less obviously as the result of the procedures involved in making microscopic preparations. The spines are composed of concentric layers of cuticular substance. Down the axis is a column of nonlaminated material, apparently the pulpy remains of the earliest layers. The laminated nature of the spines is easily seen in live material, or in preserved specimens cleared in glycerine. In balsam mounts the lamination is less distinct and often the superficial layer stains much more deeply than the interior layers.

Rare specimens of both species have been encountered with black spines. These specimens always came from dead ratfishes in which the intestinal contents were blackened and putrid. The spines evidently had absorbed the black color from the decomposing bile and food residue, since the projecting tips were much blacker than

embedded parts, and the outer layers darker than the more axial portions. Black spines cannot be considered a specific character (Haswell, 1902), and the acetabular spines of *G. fimbriata* are not typically black as stated by Watson (1911, p. 369).

(a) *Acetabular spines.*—The acetabular spines are difficult to count accurately in whole mounts. In *G. fimbriata* they rarely stain. In *G. urna* they sometimes stain distinctly with alum haematoxylin. They are arranged in imperfectly longitudinal and obliquely dorsoventral rows; thus present an imperfectly quincuncial arrangement in surface (lateral) view. In the few specimens examined in this way there were from 5 to 8 longitudinal rows in *G. fimbriata* and 4 or 5 in *G. urna.*

In *G. fimbriata* the acetabular spines (Figs. 19–23) are small, 175 (84–300) μ long by 31 (7–65) μ in maximum diameter. The shape is stubby, much like a tenpin, but often asymmetrical or clublike.

In *G. urna magnispinosa* the acetabular spines (Figs. 9–10) are extremely large, 578 (420–800) μ long by 54 (40–85) μ in maximum diameter. The shape is graceful and symmetrical. In *G. urna parvispinosa* (Figs. 14–17) the size, 250 (180–360) μ by 34 (25–55) μ, averages larger than in *G. fimbriata,* although the size ranges overlap. In shape they resemble those of f. *magnispinosa,* although less elegant and symmetrical.

There is no evidence that f. *parvispinosa* is a stage in the development of f. *magnispinosa.* It is possible, however, that a study of a large enough series of *G. urna* might reveal intergradations between the two presumptive varieties.

(b) *The body spines.*—At the posterior end of *G. fimbriata* the body spines (Figs. 24–29) encircle the funnel region, and are found quite to the base of the plications of the rosette. In a third of the specimens they are set in inconspicuous papillae. On the dorsal side they extend forward about as far as the pore of the funnel medially, but laterally may reach about as far as the level of the posterior boundary of the ovary or seminal receptacle. *Rarely,* they extend forward, diminishing in size and becoming more widely separated, to the level of the posterior part of the uterine sac. On the ventral side they rarely extend forward more than three-fourths of the way from the rosette to the level of the funnel pore, and never further anteriorly than the ovary. The average size is 148×37 μ, but they may vary from 35×12 μ to 250×50 μ. Usually they are smaller than the acetabular spines, but in 20 per cent of the specimens are somewhat larger.

In some individuals they are so minute and scattering that the worm is practically spineless. It seems possible that very few individuals of *G. fimbriata* are naturally devoid of body spines. Juvenile specimens, or even small mature ones, often have a row of closely set spines extending the entire length of the lateral margins. In mature adults these are usually reduced to a few anterolaterals and a few on the ruffles of funnel region, true marginal spines along the borders of the ruffles being very sparse or even apparently absent altogether.

In *G. urna* the body spines (Figs. 11–13, 18) encircle the body in the anterior half of the funnel region, thus do not occur as far posteriorly as in *G. fimbriata.* On the dorsal side they extend anteriorly, usually embedded in distinct elevated papillae, for 80 per cent of the length of the worm. In the median area, overlying the principal reproductive organs, they extend forward as far as the anterior end of the spiral uterus, or the posterior fourth of the uterine sac. Laterally, they extend forward for one-half to three-fourths the length of the uterine sac and may even

extend to, and merge with, the anterolateral spines. The dorsal spines occur in irregular and undulating transverse rugae, each spine on a distinct papilla. The arrangement is imperfectly quincuncial. Contrary to the statement of Watson (1911, p. 370) the "pattern" is much more distinct in *urna* than in *fimbriata*. The body spines are largest and most closely set in the posterior part of the body; anterior to the level of the ovary they become more and more widely spaced and gradually smaller.

The ventral body spines range from the middle of the funnel region to the level of, or slightly anterior to, the dorsal pore of the funnel. Laterally, they merge with the marginal spines. Often the ventral spines are so sparse as to form no distinct group.

The largest body spines, on the posterior dorsal or lateral surfaces, are about the same size in the varieties with the large and small acetabular spines; 235 (150–360) $\mu \times 50$ (22–90) μ in the former and 262 (190–325) $\mu \times 49$ (35–75) μ in the latter. In f. *magnispinosa* they are never as large as the acetabular spines; in f. *parvispinosa* they are slightly larger than the acetabular spines in about one out of five individuals.

In *G. urna* the marginal spines may be large, conspicuous and numerous the entire length of the lateral margins, or few or absent in the anterior one-half to two-thirds of the margins, although always abundant from the level of the ovary backward.

5. *The excretory pores* (*ex.p.*, Figs. 1–2).—The excretory pores are located dorsally near the junction of the thicker median and thinner lateral portions of the body, a short distance posterior to the level of the vaginal pore. In *G. fimbriata* the excretory pores are extremely inconspicuous. However, they can be located in most stained whole mounts by one familiar with their appearance and location. They are usually posterior to the level of the anterior border of the uterine sac. In *G. urna* the excretory pores are much more conspicuous, and often have a distinct swollen vesicle just beneath the pore. They are usually anterior to the level of the anterior end of the uterine sac, distinctly more anterior than in *G. fimbriata*.

6. *The uterine pore*.—The uterine pore (*ut.p.*, Fig. 2) is located on the ventral surface, in the median line, a short distance back of the anterior end of the uterine sac. It is patent and readily seen only in a minority of specimens. In percentages of the total length of the worm, the uterine pore is 18 (12–24) per cent distant from the anterior end in *G. fimbriata* and 15 (12–23) per cent in *G. urna*.

7. *Testes*.—The testes occur as a large number of spheroidal follicles arranged in two fields, right and left, in the medulla of the anterior end of the body. The fields are separated by the uterine sac, vas deferens, ejaculatory duct and part of the vagina. In about half of the specimens of both species a few testicular follicles bridge over the gap between the two fields just behind the acetabulum. The right testicular field is divided into anterior and posterior portions by the vagina which crosses it.

In *G. fimbriata* the testicular follicles are very numerous, crowded, and form deeply staining compact groups extending from the middle of the acetabulum to, or beyond, the posterior end of the uterine sac in 88 per cent of the cases, and posterior to the middle of the uterine sac in all cases.

In *G. urna* the follicles are much fewer in number, often more widely spaced, and

of smaller average size. The testicular fields extend from the posterior fourth of the acetabulum to the anterior part of the uterine sac. In 37 per cent of the specimens they reach only to the anterior border of the uterine sac; in 45 per cent they extend only one-third the length, and in 18 per cent one-half the length of the uterine sac. The compact parts of the testicular fields are relatively shorter than in *G. fimbriata,* without exception, although in rare specimens a few scattered follicles follow the course of the nerve cords for a short distance further.

8. *The male pore.*—The pore of the ejaculatory duct is located ventrally, slightly to the right of the median line. In terms of total body length, its average distance from the anterior end is 10.8 per cent in *G. urna* and 13.3 per cent in *G. fimbriata.* However, the pore is relatively much farther from the anterior end in small specimens (13.7 per cent in *G. urna;* 16.7 per cent in *G. fimbriata*) and closer in those of maximum size (8.4 per cent in *G. urna;* 9.2 per cent in *G. fimbriata*).

In both species the male pore is usually slightly posterior to the level of the vaginal pore, rarely at the same level or slightly anterior thereto.

9. *The vas deferens and ejaculatory duct* (Figs. 38–39).—As seen in whole mounts, the true length of the ejaculatory duct cannot be measured, since it slopes ventrally from its posterior to its anterior end and also curves downward and to the right. It is usually foreshortened by about 20 per cent, as seen in dorsal or ventral view. The measurements used in the following comparison are its anteroposterior extent, and not its actual length. The ejaculatory duct (and also the anterior, thickened part of the vagina) seems to be independently contractile and extensile, which probably accounts for the rather great range in its length.

In *G. fimbriata* the ejaculatory duct has an anteroposterior extent of 1.6 (1.1–2.7) mm, which is 98 (65–150) per cent of the length of the adjacent thickened part of the vagina. In *G. urna* the anteroposterior extent of the ejaculatory duct is 0.9 (0.6–1.15) mm; only 48 (32–74) per cent as long as the thickened part of the vagina. Consequently, the ejaculatory duct of *fimbriata* is nearly twice as long as that of *urna* on the average, and is also longer relative to the thickened part of the vagina.

At the fundus, or posterior end, of the ejaculatory duct, there is a papilla (*pa.,* Fig. 38) traversed by a funnel-like canal for the passage of spermatozoa from the vas deferens. In *fimbriata* the papilla is surrounded by a large bulbus propulsorius (*bu.pr.,* Fig. 38) about twice the diameter of the rest of the duct. In fifteen specimens the bulb measured 288 (165–415) μ long by 249 (165–310) μ in diameter. In *G. urna* the posterior end of the ejaculatory duct is not differentiated into a muscular bulb.

In *G. fimbriata* the vas deferens is of greater diameter, longer, more convoluted and much more capacious than in *urna.* In *G. urna* the anterior end of the vas deferens expands into a large ampulla (*a.v.d.,* Fig. 39) which may be mistaken for a bulbus propulsorius if its true position and relations with reference to the thick-walled ejaculatory duct are not noted. The epithelium lining the ampulla appears to be ciliated; possibly it should be considered to be the dilated posterior end of the valve-like papilla.

10. *The vagina and the vaginal pore.*—In *G. fimbriata* the thickened anterior part of the vagina is 1.5 (1.0–3.1) mm; in *urna* 1.9 (1.0–3.0) mm in length (average of 20 specimens of each species). This cuticle-lined, muscular section of the vagina

is usually more funnel-like in *fimbriata;* i.e., wider at its distal and more tapering toward the posterior end than in *urna* in which it is more slender and of more uniform diameter.

In *G. fimbriata* the vaginal pore (*p.vag.,* Fig. 2) is 10.7 (7.5–15) per cent; in *G. urna* 9.4 (7.1–14.1) per cent of the total length of the worm from the anterior end. As with the male pore, it is relatively closer to the anterior end in large than in small specimens. The vaginal pore is relatively closer to the lateral border in *fimbriata* than in *urna.*

11. *Vitelline glands.*—The vitelline follicles are absent, or very sparse, in the median part of the body overlying the central axis of reproductive organs. Anteriorly they are found on each side of the acetabulum, where they occur a little farther forward than the testes. From here they extend posteriorly, lateral to the axis of sex organs, both in the thicker median and the thinner lateral portions of the body, to the region between the level of the ovary and dorsal pore where they encircle the body more or less completely, although sometimes sparse medially on both dorsal and ventral sides. Throughout they are more numerous and crowded in the lateral margins, and become more widely spaced medially. The following specific difference is easily noted: In *G. urna* the vitelline follicles terminate posteriorly between the ovary and the dorsal pore of the funnel (*vit.,* Fig. 2). In *G. fimbriata* they also stop anterior to the dorsal pore medially, but extend laterally as far as the lateral ruffles; i.e., in mature specimens 1 or 2 mm posterior to the dorsal pore, on each side of the funnel (*vit.,* Fig. 1). No exceptions to this specific difference have been noted.

12. *Eggs.*—The eggs (Figs. 30–31) are plumply ellipsoid with bluntly rounded ends. Often the operculate end is slightly blunter than the other. In freshly deposited eggs the operculum cannot be seen, although the suture can be revealed by the application of acetocarmine, and it often can be detected in eggs which have stood in a salt solution for several days. The thin amber-colored shell is surrounded by a layer of jelly which remains invisible unless India ink is mixed with the medium in which the eggs are being examined. About 10 per cent of the eggs have a minute boss at the end opposite the operculum. As compared with those of *G. fimbriata,* the eggs of *G. urna* are slightly more pointed at the ends, i.e., shaped more like a Rugby football; have slightly thinner shells, and a slightly thicker layer of jelly. However, the differences between the eggs of the two species are so trivial that they cannot be readily used for specific determination.

Of the specific differences listed above, the relative lengths of the testicular fields, the size and complexity of the rosette, the distribution and appearance of the body spines, the size and grouping of acetabular spines (at least in *G. urna magnispinosa*), the size and appearance of vas deferens and ejaculatory duct, and the posterior limit of the vitelline glands are without interspecific gradations. Although their ranges overlap, the remaining characters are useful in the identification of specimens. In the opinion of the writer, it is not possible to confuse the species of *well-extended* preserved specimens, although the extreme mobility and contractility of the living worms result in occasional perplexity on the part of the observer.

VI. DISPUTED POINTS IN THE MORPHOLOGY OF *Gyrocotyle*

1. *Excretory pores.*—The excretory pores are visible in 86 per cent of my whole mounts of *G. urna* and in 78 per cent of those of *G. fimbriata.* Not only the pores,

but the large excretory vessels leading to them are often visible. The pores are easily found in serial cross-sections of either species. Spencer (1889) was the first to observe the true excretory pores. His illustration correctly depicts their location. Linton (1924) found the excretory pores and localized them correctly. Fuhrmann (1930–31, p. 161 and 171) states that the excretory pores are *ventral,* but otherwise localizes them correctly. Since Spencer's original discovery Lönnberg (1891), Hungerbühler (1910) and Watson (1911) were unable to find the pores. Dollfus (1923, p. 205, footnote 3 and p. 210, footnote 1) even doubts Spencer's observation and tries to homologize the acetabulum with the posterior excretory pore of the Digenea and certain Cestodes.

2. *Origin of the funnel by closure of the borders of a groove.*—Lönnberg (1891, p. 29) advanced the theory that the funnel, with its distal and proximal openings, was formed phylogenetically by the dorsal approximation and partial fusion of the lips of an elongated groove such as the bothrium of a Pseudophyllidean Cestode. Watson (1911, p. 428) seems to accept this hypothesis as a demonstrated fact. Ward (1912, p. 731) likewise appears to accept the theory as a fact and even describes the rosette as having a bilateral morphology, and states that the distal plicated border is horseshoe-shaped, with a ventral notch and furrow dividing it into a right and left series of ruffles continuous dorsally (Ward, 1912, p. 729 and p. 731).

My material of *Gyrocotyle fimbriata* includes numerous minute postlarvae in which the rosette is just beginning to appear, hundreds of juveniles with the rosette in all stages of development and adults of all sizes and degrees of maturity. There is no indication at any stage of a groove, the edges of which fuse to form a funnel. There is no sign, at any stage of development, of a bilateral arrangement of the plications of the rosette. In early stages of development the rosette has the form of a circular flange growing out from around the body just anterior to the hook-bearing process of the posterior end (Figs. 6–8). The border of the rosette remains circular until the development of plications begins to distort its symmetry, but at no time do the plications arrange themselves in any uniform or bilateral pattern, and at no time is the wall of the funnel divided longitudinally by any kind of notch or furrow. The dorsal pore appears as such, just anterior to the base of the papilla bearing the larval hooks, and has been discerned in a few specimens as small as 1.8–2.6 mm in length. Whatever its phylogenetic origin, there is nothing in the morphology or ontogeny of the funnel to support Lönnberg's theory.

3. *The genital notch (ins.gen.,* Figs. 1–2).—The term "genital notch" has been applied to the indentation of the right margin which appears to be the termination of a groove proceeding from the vaginal pore. The notch is absent from 8 per cent of the preserved specimens of *G. fimbriata* and from 28 per cent of those of *G. urna.* It is usually deeper and more conspicuous in the former species. Ward (1912, p. 725) states that the genital notch occurs on both the right and left margins of the body. As a matter of fact, a scallop or indentation on the left side opposite the genital notch, appears to be merely a portion of the undulant margin, and not a counterpart of the genital notch, and such is entirely lacking from 66 per cent of the *G. fimbriata* and 44 per cent of the *G. urna* in the collection of the writer. In *urna* a distinct groove from the vaginal pore to the notch (or margin) is absent from 61 per cent, in *fimbriata* from 35 per cent, of the specimens.

4. *Suppression of lateral ruffles and rosette by muscular action.*—Lönnberg

(1891) describes *G. urna* as elongating to the extent that the lateral undulations completely disappear, and even the plications of the rosette in extremely elongated specimens. Olsson (1896) depicts specimens in the same extended state without obvious lateral undulations or rosette. Fuhrmann (1930–31, p. 170) states that the lateral ruffles are formed mechanically by muscular contraction. Dollfus (1923, p. 232, footnote 2) affirms that the plications of rosette and body margins may disappear completely in movements of elongation. Ward (1912, p. 727–728) states that the extent and complexity of the lateral ruffles depend on the state of contraction, and implies that they may disappear completely upon elongation of the worm.

The writer has observed small, scarcely mature specimens of *G. urna* elongate themselves to such an extent that the lateral undulations completely disappear, but without complete disappearance of the folds of the rosette. The observations of Lönnberg and Olsson are probably correct, but it is likely that they apply only to small specimens. The writer doubts that the lateral undulations of a mature, fully grown *G. urna* can be suppressed by elongation of the body, and feels certain that full-grown *G. fimbriata* is incapable of eliminating the folds of the lateral margins, or of the rosette, by any degree of extension.

5. *The male reproductive ograns* (Figs. 38–39).—Lönnberg (1891) states that the ejaculatory duct has a ciliated lining except in the terminal part. Watson (1911, p. 379) states that the ejaculatory duct is lined with spinules. Haswell (1902) states that the ejaculatory duct is lined with chitinous denticles in *G. urna* and *G. nigrosetosa.*

The nature of the lining of the ejaculatory duct both in *G. fimbriata* and in *G. urna* is as follows: Beginning at the posterior end, the flattened epithelium covering the papilla and lining the muscular bulb, or fundus of the duct, is ciliated. The ciliated epithelium extends through the canal of the papilla in both species and into the terminal ampulla of the vas deferens in *urna.* The middle portion of the duct, which receives the secretion of the innumerable prostate gland cells, is lined by a low columnar epithelium which is not ciliated although the cells are vacuolated and the borders often frayed or seemingly macerated (coagulated secretion of the prostate gland?). Approximately the distal fifth of the ejaculatory duct is lined by cuticle continuous with that of the surface of the body. No spinules are present.

Spencer (1889) described the male pore as opening close to the margin on a conical papilla. He thought the ejaculatory duct is eversible. Although he called his specimens *G. urna,* his description was probably based upon *G. rugosa.*

Haswell (1902) stated that the male pore of his specimens of *G. rugosa* was situated on the apex of a papilla large enough to be curved around the margin of the body to the dorsal side, but that a similar papilla was not obvious in *G. urna.*

Lönnberg (1891) looked upon the proximal papilla ("Zapfen") as a valve to prevent the sperm discharged into the ejaculatory duct from returning to the vas deferens. He termed the entire ejaculatory duct the "penis apparatus" and stated that the external aperture lies on a papilla in a circular depression of the ventral surface. He thought that the terminal papilla functions as a penis, and that the terminal part of the ejaculatory duct is evaginable.

Watson (1911) designated the papilla at the posterior end of the ejaculatory duct the "penis papilla," and the ejaculatory duct the "cirrus pouch." She found the male pore to be located on a rounded papilla. She made no surmises regarding the functions of either papilla.

Fuhrmann (1930–31) looked upon the posterior papilla as a penis and appeared to think that the entire ductus ejaculatorius can be everted with the proximal papilla at its apex. He compares it with the protrusible penis of the TURBELLARIA and the MONOGENEA.

Here are two contrary opinions: one that the papilla at the proximal end of the ejaculatory duct is a protrusible intromittent organ, the other that the distal or terminal papilla bearing the genital pore is an intromittent organ. In the hundreds of specimens examined by the writer there is no evidence that the valvelike papilla in the fundus of the ejaculatory duct is ever protruded, or that it can even approximate the external opening, and no specimen was seen with the slightest eversion of the ejaculatory duct. The proximal part of the cuticle-lined terminal portion is sometimes prolapsed into the wider opening at the surface, thus forming a papilla (Fig. 39). There appears to be no permanently differentiated penis, although it is likely that the end of the ejaculatory duct and the surrounding surface may be protruded to form a temporary intromittent organ.

6. *The female genital complex.*—Apparently only Spencer (1889), Hungerbühler (1910) and Watson (1911) have attempted a complete analysis of the female reproductive system. Spencer's diagram omits the shell gland, yolk reservoir and ovicapt, and is excessively schematized. Hungerbühler presents a diagram which is essentially correct, but so schematized that it fails to show the true shape and proportions of the organs involved, and from which the ovicapt and shell gland are omitted. He erroneously states that the ductus seminalis is connected with the egg receptacle. Watson describes the female organs at length, but fails to describe the ovicapt, depicts the yolk reservoir with entirely erroneous shape and relations, and neglects to state where the ducts of the shell gland terminate.

Description of the Female Genital System in G. urna *and* G. fimbriata

(Figs. 36–37)

The vagina extends from the vaginal pore, located anteriorly and dorsally, near the right border, down the right side of the uterus to open ventrally on the right anterior, or rarely the left anterior, side of the receptaculum seminis. In large specimens with highly developed uterine coils the vagina may be threaded between the loops of the uterus and be difficult to trace, even in serial sections. The anterior 5 to 13 per cent of the vagina in *fimbriata,* or 5 to 18 per cent in *urna,* depending on the degree of contraction or extension, is thick-walled and lined throughout by cuticle. A very narrow canal connects its lumen with the remaining thin-walled part of the vagina which is lined by a low, nearly cuboidal epithelium, which is ciliated in its posterior fourth.

The receptaculum seminis is nearly spherical, although usually longer than broad, and slightly flattened dorsoventrally. In mature worms its longitudinal and transverse diameters range from 0.45×0.37 mm to 2.2×2.0 mm. It is crowded with spermatozoa, and often contains scattering ova and vitelline cells. In immature specimens it is smaller, and often appears like a pyriform enlargement of the posterior end of the vagina.

The afferent oviducts, coming from the horseshoe-shaped aggregation of ovarian follicles, converge to a receptaculum ovorum on the ventral side of the seminal

receptacle. The number and arrangement of oviducts is inconstant. The egg receptacle is dorso-ventrally flattened, transversely elongated, and is of various sizes and shapes in different specimens. In large worms of either species it may be from 300 to 700 μ in transverse diameter and from 100 to 200 μ in antero-posterior diameter. Its posteromedian border opens into a conspicuous muscular ovicapt which continues posteriorly as the ciliated efferent oviduct, or fertilization canal. The short ductus seminalis, coming from the seminal receptacle, unites with the efferent oviduct just distally to the ovicapt. Typically, the ductus seminalis lies dorsally to the ovicapt; their superimposed position often results in the two ducts appearing as one in whole mounts or frontal sections.

The yolk reservoir empties into the oviduct just distal to the junction of the seminal duct therewith. The yolk reservoir is extremely conspicuous; indeed, it was correctly described and illustrated by Wagener (1852). Its shape is that of a spindle bent into a crescent, or a short helicoid curve, with a variable number of yolk ducts emptying into its more anterior and ventral end. It is invariably crowded with opaque yolk cells. It varies from 200 to 700 μ in length by 70 to 100 μ in greatest diameter. After its junction with the yolk reservoir, the oviduct begins to curve, usually to the left, occasionally to the right (where the vagina enters the seminal receptacle on the left side), and enlarges somewhat both in the diameter of the lumen and the thickness of the walls. At this point, near the posterior boundary of the seminal receptacle, the ducts of the shell gland penetrate the walls of the oviduct. This portion of the oviduct has all the characteristics of an oötype, and is here considered to be such, although it is invariably empty in preserved specimens.

Distal to the oötype the oviduct, now better termed the uterine duct, contracts a little in diameter and continues anteriorly in a sinuous course to the anterior boundary of the seminal receptacle, where it becomes so excessively coiled and intertwined that its true length is hard to judge. The proximal part contains scattering ova, yolk cells and secretory globules; the more convoluted distal portions fully formed eggs. As the eggs accumulate, the uterine duct rather quickly enlarges, loses its cilia, and passes over into the uterus.

The fact that in my preparations the oötype is invariably empty, and the proximal parts of the uterine duct contain only the constituent elements which go to form the compound egg, at first led to the supposition that the egg is actually formed in the more distal parts of the uterine duct. Watson (1911, p. 397) says, "The first two or three coils of the uterus . . . may be regarded as an oötype . . . ," meaning, apparently, that the entire proximal portion of the uterine duct functions as an oötype. She also observed fully formed eggs only in the convolutions of the uterus anterior to the receptaculum seminis. The probable explanation of the emptiness of the oötype and proximal part of the uterine duct is that the worms are usually fixed hours, or days, after removal from the host and that normal egg production had long since ceased.

In both species the uterus consists of two parts, a proximal spirally coiled portion and a terminal saccate portion, for convenience designated the *uterus* and *uterine sac*. The uterine sac comprises 35 (24–54) per cent of the total length of the uterus. The uterine pore opens in the mid-ventral line a short distance back of the anterior end of the saccate uterus. It is often invisible in small mature worms, and probably becomes patent only after a large quantity of eggs has accumulated. The uterus

and uterine sac are relatively bulkier, with a greater volume of eggs, in *G. fimbriata,* but otherwise there is no distinct difference in the morphology or proportions in the two species. Among previous students of the genus, only Watson seems to have distinguished clearly the uterine sac as distinct from the rest of the uterus.

7. *The nervous system.*—Only Watson (1911) has attempted a detailed analysis of the nervous system of *Gyrocotyle.* The writer will only present a few observations which differ from the interpretations of Watson.

Miss Watson describes the two large posterior ganglia ("ganglionic knots") as connected by a "posterior bridge commissure." Each ganglion is said to divide at its posterior end into dorsal and ventral branches which pass medially to join similar branches from the opposite side to form a "proximal ring commissure." Further posteriorly, near the base of the rosette she found a "distal ring commissure" connected with the "proximal ring commissure" by four connectives, two from the dorsal and two from the ventral portions thereof, and with the ganglia by a single nerve on each side which bifurcates before uniting with the "distal ring commissure."

Fig. 35 is a drawing of the posterior ganglia and commissures from a whole mount of *G. urna.* The same pattern is found in *G. fimbriata.* It will be noticed that the posterior transverse commissure (Fig. 35, *comm.p.*), between the anterior ends of the two large posterior ganglia, bears a pair of distinct commissural ganglia not mentioned by Watson. At their posterior ends, the two main ganglia divide each into three branches (Fig. 35, *ra.a., ra.m., ra.v.*) of which the dorsal and ventral ones proceed medially and anastomose with the branch from the opposite side, while giving off numerous anastomosing twigs towards the posterior end. The posterolateral ramus proceeds much further posteriorly before dividing into ramifying twigs.

Watson's description of the posterior ganglia and commissures is correct in the main, but deficient or obscure in the following details: 1. She failed to observe the two ganglia of the posterior commissure. 2. She implies that there is a continuous ring commissure near the ganglia, although in reality she describes dorsal and ventral semicircular commissures. Actually, there is no continuous "proximal ring commissure." 3. Her drawing (Watson, 1911, pl. 35, fig. 17) is obscure and does not show the posterolateral rami as distinct from the dorsal and ventral posterior rami. 4. The distal ring commissure described by Watson cannot be seen in whole mounts and I have been unable to trace it in serial sections. The numerous subdivisions of the three posterior rami proceeding from each ganglion anastomose to form an elaborate network, which encircles the posterior part of the funnel region, and extends into the plications where its ultimate ramifications cannot be traced. A well-defined commissure, sharply delimited from the network, and forming a continuous ring proximal to the folds of the rosette, obviously does not exist.

The writer has been unable to discern the sensory ridges and sensory pits of the acetabulum, described by Watson, in living worms, whole mounts or in serial sections.

VII. LIFE HISTORY

Since *Gyrocotyle* is a parasitic Platyhelminth and has been regarded both as a Trematode and a Cestode, it has generally been assumed that it has one or more intermediate hosts. The manner in which the Chimaerid fishes receive their infestations is still unknown. Except for *G. rugosa,* the eggs of which contain a fully

developed larva at the time of deposition, the eggs are discharged in early embryonic stages and must undergo several weeks of development before hatching.

Ruszkowski (1932) and the writer have both kept eggs in dishes of sea water until the free-swimming larvae hatch, so it is logical to believe that in nature the larvae develop in eggs which have passed out of the intestine of the host and that they penetrate some intermediate host on the ocean floor, although this belief is without supporting evidence.

Fig. 5 is a free-hand drawing of an actively swimming decacanth larva of *G. fimbriata.* Most swimming specimens are slightly curved toward one (the ventral?) side. As Ruszkowski pointed out, the larvae swim with the openings of the glandular ducts at the anterior end and with the cluster of larval hooks at the posterior end. Since the rosette and funnel develop at the end with the larval hooks, he was able definitively to settle the oft-debated question as to which end of the adult actually is anterior.

It is extremely difficult to get exact measurements of the living larvae of *Gyrocotyle* and to analyze the internal structures. When in good condition they are in constant, rapid motion. If restrained in a hanging drop or under a cover glass they quickly contract into various spheroid, ovoid, or irregular shapes and soon the entire anterior half disintegrates into a mass of globules and protoplasmic debris. In most fixing fluids they contract to an ovoid shape. Specimens discharged from a pipette into a mixture of five parts of 6 per cent formalin and one part of 2 per cent osmic acid contracted only slightly, and died at an average length of 140 μ by 40 μ in maximum diameter. Normal living specimens have about the same length, but seem more slender. The larvae are extremely contractile and extensile, and normally active ones may elongate to about 170 μ with a diameter of about 20 μ, or may contract to dimensions of about 100 μ by 30–40 μ.

Typically, they have a short fusiform shape, with the greatest width from 20 to 50 per cent of the total length from the anterior end. The entire larva, except for the extreme anterior end, is covered with a dense layer of cilia which, when actively beating, form a layer about 5 μ thick. In dead or moribund specimens the cilia stand out at right angles to the surface and appear to be about 11 μ long, except near the anterior end where they become progressively shorter and disappear. The anterior end, like the corresponding area of a miracidium, is devoid of cilia and is protrusible and retractile. The cilia arise from a thin membranous epithelium about one micron thick, in which scattered nuclei are present, although cell boundaries could not be distinguished. Immediately beneath the epithelium are circular myonemes, more numerous anteriorly than posteriorly.

Terminating at the anterior end are two long ducts which stain intensely with neutral red or nile blue A and which appear to be filled with a nearly solid secretion. They can be traced posteriorly about two-thirds the length of the larva, but become indistinct and fade from view just anterior to a mass of vaguely outlined cells located in front of the larval hooks. The two ducts are closer to one surface (arbitrarily considered dorsal) than to the other. At the anterior end they seem to terminate separately in some, and to unite in other, larvae. A short distance back from the anterior end is a large bilobed mass of cells which may possibly be the "brain" or Anlage of the nervous system. The parenchyma of the entire larva contains numerous refractive globules, mostly from 2 to 5 μ, but ranging from less than

one to 10 μ in diameter. These globules are clustered in large numbers ventral to the "brain" and near the posterior end of the secretory ducts. Occasionally, two small transient clear vesicles, right and left, could be seen anterior and lateral to the "brain"; possibly contractile excretory vesicles.

The ten larval hooks are disposed in a circle at the posterior end, completely retracted within the body, with the tips turned inward and the manubria diverging. The hooks are protruded so infrequently that an exact analysis of the process was impossible. Apparently, the ciliated integument is retracted at the same time that a pluglike papilla bearing the hooks is protruded. The curved tips of the hooks thus appear spread around the periphery of the papilla, with the manubria converging toward the longitudinal axis of the larva. Upon withdrawal of the papilla, the tips close in toward the center.

The larval hooks (Figs. 32–34) are 21 (19–22) μ in length. They persist in postlarval stages at the free end of a pluglike papilla and apparently are functional in the smaller juvenile specimens. After the formation of the dorsal pore and funnel, the hooks become incorporated in the dorsal wall of the funnel where they occasionally can be seen in fully mature specimens. In fact, they can clearly be seen in one of my whole mounts of *G. urna* 32 mm in length.

The following zoologists have reported finding postlarvae in various stages of development in the parenchyma of larger specimens of *Gyrocotyle:* Hungerbühler (1910) in *G. rugosa* and *G. urna;* Linton (1924) in *G. plana* (= *G. rugosa*), and Fuhrmann (1930–31) in *G. urna* and *Gyrocotyloides nybelini.*

In the course of this study such embedded larvae have been found in the parenchyma of 15 adult specimens of *G. urna,* in 23 adults of *G. fimbriata* and in 6 small juvenile *fimbriata* ranging from 0.9 to 7.0 mm in length. The embedded postlarvae measure from 110 × 50 μ (scarcely bulkier than a free-swimming larva) to 1800 × 380 μ. They lie at various depths beneath the surface, in any part of the host, but are commonest just anterior to the uterine sac and in the vicinity of the male genital pore.

In two specimens of immature *G. urna,* the uterine sac (as yet devoid of eggs) was occupied by a juvenile *Gyrocotyle,* one 2.1 mm long, the other 6.9 mm long—longer than the uterine sac and consequently folded back on itself at either end. Since an open uterine pore could not be discovered in either case, it is likely that the ensconced juveniles had developed to their relatively large size in their actual location.

Immediately in front of the uterine sac, on the ventral surface of the worm, is a subcircular area of closely crowded nuclei, 250 to 500 μ in diameter, with thickened cuticle, which usually has a depression in its center. It is more conspicuous and well defined in *G. urna* than in *G. fimbriata.* Of the 40 mature specimens of both species with embedded postlarvae, 23 had all, or part, of the postlarvae lodged beneath, or adjacent to, this spot. Only Wagener (1852, p. 552) seems to have noted the spot. Since its exact function is unknown, it is given the non-committal name of *fovea Wageneri* in this paper (*fov.wag.,* Figs. 1 and 2).

The maximum number of postlarvae found in any one host was 101, in a specimen of *G. fimbriata* 26 mm long. Usually, only from 1 to 8 are present; in fact, 76 per cent of the infected host animals harbor only one parasitic postlarva.

The considerable frequency with which normally developing postlarvae are

encountered in the tissues of larger *Gyrocotyle,* which thus function as nurse animals, suggests that this is not an abnormal or chance occurrence, but a normal phase in the life history of the genus. On the other hand, as Fuhrmann suggested, they may normally pass through early postlarval stages in the intestinal mucosa of the ratfish and only enter the larger *Gyrocotyle* incidentally, or under abnormal conditions.

VIII. PERCENTAGE OF INFESTATION AND THE NUMBER OF *Gyrocotyle* IN A SINGLE HOST

Most writers on *Gyrocotyle* have reported that there normally are only one or two worms in a single host, although rarely several may be found. My records do not indicate precisely the percentage of infestation nor the correct number of worms in the host in every case, since often worms escape from the host before it can be examined for parasites. In the course of this study, 20 specimens of *Gyrocotyle* have been collected from the bottom of containers in which ratfishes were transported. The following figures are of some significance, however, in view of the large number of ratfishes examined.

Data are available for 167 ratfishes. Of these, 24 were negative for *Gyrocotyle,* although possibly infested when first captured. Of the 143 infested ratfishes, 3 harbored *G. fimbriata* and *G. urna* simultaneously; 104 had *G. fimbriata* only and 36 had *G. urna* only.

Of the 104 ratfishes with *G. fimbriata,* 16 had one worm; 70 had two; 6 had three; 1 had five; 4 had six; and 7 had mass infestations of large numbers of small juvenile worms, ranging from 7 to 203 in a host.

Of the 36 ratfishes with *G. urna,* 8 had one worm; 26 had two, and 2 had three.

These records agree with the experiences of others, and show that the ratfish usually has two mature *Gyrocotyle,* of one species, in the spiral valve. The manner in which the large number of juvenile worms sometimes present is reduced to several adults, or how the presence of one species excludes the other, remains to be elucidated.

IX. THE TAXONOMIC POSITION OF THE GENUS *Gyrocotyle*

Gyrocotyle is classified as a Cestode primarily because of the lack of a digestive tract, although the pattern of the reproductive system, with its large ovicapt and long uterine duct, bears some resemblance to that of the TETRARHYNCHIDEA. On the other hand, the anterior sucker and posterior haptor, the position of the excretory pores, the anteroventral uterine and male genital pores, and the dorsal vaginal pore (cf. *Microcotyle, Hexacotyle,* etc.) lend it the superficial appearance of a Monogenetic Trematode. There obviously is no series of morphologically different larval stages strictly comparable to the procercoids, plerocercoids, etc., of Cestodes, and as yet no positive evidence that intermediate hosts are involved in the life cycle. The free-swimming larva resembles no known cestode larva with the possible exception of the poorly described larva of the Amphilinidae. To judge from the postlarval stages found in the intestine of the ratfish, or embedded in the tissues of adult *Gyrocotyle,* the metamorphosis from the larval to the adult stage is extremely simple and direct, and much more comparable to that of the Monogenea than to that of the Cestoda. Until the life history of the genus has been completely elucidated its taxo-

nomic position will remain obscure. It can be considered a Cestode only provisionally.

X. THE VALID SPECIES OF *Gyrocotyle*

The reader is referred to the works of Watson (1911) and Dollfus (1923) for a review of the complicated bibliography of *Gyrocotyle* and summaries of the contents of early publications.

Gyrocotoyle rugosa *Diesing, 1850; the type species.*—The first species named was *G. rugosa,* described by Diesing in 1850, and redescribed with illustrations in 1855. His description, confined to the external morphology, indicates a worm without lateral undulations, but with a fairly complicated rosette. There is no mention of spines. Diesing was in error as regards the host which was discovered by Monticelli (1889) to be *Callorhynchus antarcticus.* Descriptions of *G. rugosa* remain incomplete and unsatisfactory to the present day.

Spencer (1889) described what he believed to be *G. urna* on the basis of four specimens from *C. antarcticus.* A study of his description shows that he published a composite description of one specimen of *G. rugosa* and of three others which possibly were *G. urna.* He gives no measurements and his more important drawings apparently are of compressed and distorted specimens. He depicts the male genital pore as marginal and the uterine pore to the right of the median line.

O. v. Linstow (1901) published a brief description of a single specimen of *G. rugosa,* but was in error as regards the host. His specimen had no lateral undulations, and he saw no spines. The eggs contained a larva with 10 hooks.

Haswell (1902) added some data on *G. rugosa.* He stated that the male pore is marginal or submarginal, with a protrusible penis; that the vaginal aperture is dorsal, to the right of the median line, but posterior to the male opening. He mentioned the "embryo" with large hooks inside the egg. He did not state the host of his specimen.

Hungerbühler (1910) had seven specimens from *C. antarcticus.* He mentions lengths of 42 to 96 mm, gives measurements of breadth, thickness and width of rosette, states that the lateral margins are smooth, that transverse rugae encircle the body and that the uterus opening is median. He does not localize the male and vaginal pores precisely, but the male pore is apparently closer to the margin than in other species, at the apex of a well-developed papilla. The acetabular spines of his specimens are 200 to 300 μ long by 20 μ wide. Body spines were not observed. The egg contains a 10-hooked larva.

Linton (1924) described two specimens from *C. antarcticus* which he designated *G. plana* n. sp., but which appear to have been *G. rugosa* (this opinion was first expressed by Johnston, 1934). He described the margins as finely crenulated, but not ruffled. He observed only acetabular spines, 400 μ long by 48 μ in diameter. The male genital pore was a little to the right of the median line on a mound-like elevation, the genital and uterine pores being arranged as in *G. urna.* Testes extended 23 per cent of the total length of the worm in one specimen. Eggs in the terminal part of the uterus contained 10-hooked larvae. Only the uterine structure (a central axis with lateral diverticula) seems to distinguish this species from *G. rugosa,* and this feature probably was a misinterpretation of the uterus in strongly compressed and poorly preserved specimens.

MacDonagh (1927) briefly described two specimens of *G. rugosa* from *C. antarcticus,* fixed under pressure. Aside from measurements, his descriptions state little beyond the fact that the lateral undulations are absent, the folds of the rosette relatively simple, and spines apparently lacking.

G. rugosa undoubtedly is a distinct species. All the existing descriptions are inadequate or based on distorted specimens, and the only characters which seem to set it off clearly from other known species are the thin-shelled eggs containing a fully developed decacanth larva prior to deposition, the lack of definite ruffles on the lateral margins, the relatively smaller rosette, and the lack of body spines. The existent data on such characters as the relative position of the genital pores and the supposedly large male sex papilla are confusing and contradictory.

By combining the consistent features of the several descriptions, and omitting contradictory or dubious data, a provisional, although incomplete, diagnosis of the species can be assembled:

Gyrocotyle rugosa Diesing, 1850

Synonymy: Amphiptyches rugosa Spencer, 1889; *Gyrocotyle plana* Linton, 1924.

Diagnosis: With the characters of the genus. Mature specimens 42 to 96 mm long. Rosette relatively small and simple, about one-third (21–38 per cent) of greatest diameter of the body. Margins of the body without undulations, but transverse rugae encircle the body producing marginal crenulations. Acetabular spines 200–400 μ long by 20–48 μ in diameter; body spines probably absent. Testicular areas about 23 per cent of total length of worm. Male genital pore ventral, distinctly to right of median line, usually on a distinct papilla. The genital pores and uterine pore apparently closer to the anterior end than in other species. Eggs very thin-shelled, 96–130 μ × 52–65 μ, containing a fully developed decacanth larva at time of deposition.

Host: Callorhynchus antarcticus Lacépède.

The species of Gyrocotyle *from* Chimaera monstrosa.—An examination of the publications of European writers who have described *G. urna* from *Chimaera monstrosa* is enough to convince one that they were dealing with two distinct species. In fact, Prof. Collet of Christiania (*in* Lönnberg, 1891, p. 17) states that he believes there are two species in *Ch. monstrosa.* Lönnberg (1891) mentions a flat *Ligula*-like form and a ruffled form, but regards them as different stages in the contraction of the same species.

In spite of the voluminous European literature on *G. urna,* none of the authors has presented really good drawings of the worm, none of the descriptions contains adequate measurements of the spines, if, indeed, any measurements are given, and the distribution of the spines is usually insufficiently analyzed. The extents of the areas of testicular and vitelline follicles are usually ignored or only vaguely described. Only Wagener (1852) mentions the length of the testicular areas relative to the total length of the worm, but with no indication of how many worms were so measured. Drawings of the rosette are often so poorly executed that it is impossible to determine whether it is of the *urna* or of the *fimbriata* type. No one gives the relative breadth of rosette to breadth of body, and no one presents data on the number of lateral ruffles and their amplitude. Only the following papers contain descriptions or illustrations of any value for judging the species of *Gyrocotyle* in *Ch. monstrosa:* (1) Wagener, 1852, pl. 14, fig. 1, *urna* type; pl. 14, fig. 3, *fimbriata* type; pl. 14, fig. 7, apparently a composite drawing, although possibly *urna.* (2) Krøyer, 1852–53, gives an inadequate description, applicable only to *G. fimbriata* as far as it goes. (3) Lönnberg, 1891, pl. 3, fig. 34, *urna* type; pl. 3, fig. 36, *urna* type;

pl. 3, fig. 37 has the proportions of a juvenile *G. fimbriata.* (4) Scott, 1911, published magnified photographs of one immature and three mature specimens, which all have the precise aspect and porportions of *G. fimbriata.* (5) Dollfus, 1923, published drawings (p. 238, fig. 7) labeled *G. urna* which actually are *G. fimbriata.* (6) Fuhrmann, 1930, published a drawing (p. 161, fig. 193, 1) of a contracted specimen designated *G. urna* but apparently *G. fimbriata.*

Since the original description of *G. urna* by Wagener is obviously a composite description of two species, the problem of deciding which should retain the original specific name arises. Watson (1911) in her description of *G. fimbriata* from *Hydrolagus colliei* points out the existence in the same host of another species which she considers to be *G. urna.* Since hers is the first description which differentiates between worms of the *fimbriata* and *urna* types, her allocation of these species should be allowed to stand, although the European descriptions and illustrations involve a worm of the *fimbriata* type probably more frequently than they do the species referred to *urna* by Watson.

Gyrocotyle urna *from other hosts.*—Haswell (1902) described *G. nigrosetosa* from *Chimaera ogilbyi* Waite. The color of the spines, as pointed out above, cannot be considered a specific character. The description is inadequate for specific recognition, but such as it is, it agrees fairly well with the morphology of *G. urna.* Yamaguti (1934) reports *G. urna* from the same host. His identification is probably correct, although the description is insufficient for positive recognition. It seems highly probable that *G. urna* occurs in *Chimaera ogilbyi.*

Spencer (1889) and Hungerbühler (1910) described specimens from *Callorhynchus antarcticus* which were designated *G. urna.* The descriptions are inadequate and the presence of *G. urna* in this host is in need of further investigation.

XI. SPECIES INQUIRENDAE

Gyrocotyloides nybelini Fuhrmann, 1930. At first impression this species appears to constitute a distinct genus. It differs from *Gyrocotyle,* according to Fuhrmann, in lacking spines and lateral undulations, in having the funnel region extremely elongated and without a plicated border, in having a mere pit in place of the muscular acetabulum, testicular fields which extend back to the ovary and paired excretory pores on the lateral margins. Fuhrmann considers the elongated forms of *G. urna* described by Lönnberg to belong to his new genus.

However, Fuhrmann presents no genuine diagnosis of the genus or species. He gives no measurements of any kind, no description or drawing of the egg, and no adequate statement of the proportions and location of many internal structures. The one detailed drawing (p. 164, fig. 196) is obviously an immature specimen with the uterus still straight and undifferentiated, with the ejaculatory duct and male pore not clearly depicted, and with excretory pores, vitelline glands and nerve ganglia omitted. The number of specimens at his disposal is not stated. Thus, the description is incomplete and unconvincing. It is difficult to eliminate the suspicion that Fuhrmann was dealing with small, barely mature specimens of one of the species of *Gyrocotyle* in an unusual state of elongation.

Gyrocotyle maxima MacDonagh, 1927. The inadequate description, based on a single specimen, includes only a few external measurements. Spines were not observed. Eggs are as in *G. rugosa.* The host (*Mustelus asterias*) is probably incidental, since sharks are known to prey upon Chimaerids.

XII. SUMMARY AND CONCLUSIONS

1. It is pointed out that *Chimaera monstrosa* harbors at least two species of *Gyrocotyle,* one of which Watson considered to be the original *G. urna* and one similar to her *G. fimbriata.* Existing descriptions by European zoologists are mostly composite, as well as inadequate, and fail to distinguish between the two. It is probable that the two species from *Hydrolagus colliei* are identical with those from *Ch. monstrosa.*

2. *Gyrocotyle fimbriata* Watson from *Hydrolagus colliei* is redescribed.

3. *Gyrocotyle urna* from *Hydrolagus collici* is described, and two forms, differing in the size of the acetabular spines, are distinguished.

4. Previous descriptions of the male and female reproductive organs of *Gyrocotyle,* and of the posterior ganglia and commissures, are supplemented with added details.

5. Various controversial points in the morphology of *Gyrocotyle* are discussed and in part clarified.

6. The decacanth larva and early postlarval stages are described and illustrated. The frequent occurrence of developing postlarvae in the tissues of older individuals of the same species is noted. The existing evidence seems opposed to the theory that intermediate hosts are involved in the life cycle, although the complete life cycle remains unresolved.

7. *Gyrocotyle rugosa* is discussed and a provisional specific diagnosis is given.

8. Although existing data are inadequate for a positive decision, it is very likely that *G. urna* occurs in *Chimaera ogilbyi.* The existence of *G. urna* in *Callorhynchus antarcticus* is probable, but the data are even more unsatisfactory.

9. *Gyrocotyle maxima* MacDonagh and *Gyrocotyloides nybelini* Fuhrmann are so imperfectly described that their systematic status remains in question.

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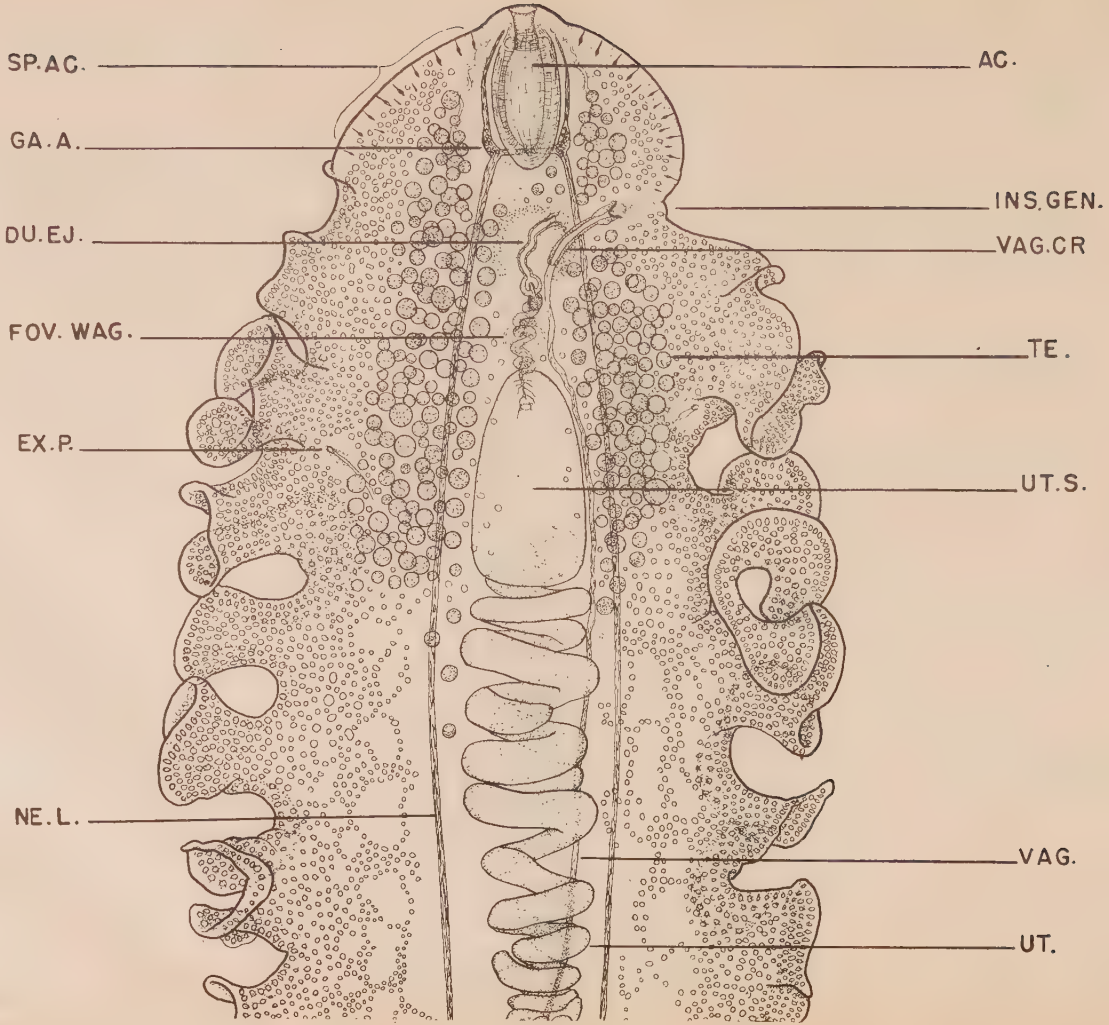
Explanation of Plates

Figs. 1 and 2 are drawings of stained whole mounts, slightly schematized as follows: (1) The mass of opaque eggs has been omitted from the uterus and uterine sac. (2) The testicular follicles are more numerous and crowded in both species than here depicted, although the relative appearance of the testicular fields in the two species is shown with approximate correctness. (3) The vitelline follicles are represented as clear circles. Each follicle is composed of a group of strongly staining cells crowded with yellowish yolk granules. Consequently, in stained preparations, the yolk follicles are semiopaque. (4) The spines, in haematoxylin-stained preparations, may vary from colorless to purple. They are here depicted as black to avoid confusion with adjacent structures.

Figs. 6–35, inclusive, were drawn with the aid of a camera lucida

Abbreviations

AC. ..... acetabulum
A.V.P. ..... ampulla of vas deferens in *G. urna*
BU.PR. ..... bulbus propulsorius of ejaculatory duct in *G. fimbriata*
COMM.P. .... posterior commissure, bearing two commissural ganglia
CU. ..... cuticle
D.P. ..... dorsal pore of funnel
DU.EJ. ..... ejaculatory duct
DU.OV. ..... oviduct
DU.SEM. .... ductus seminalis
DU.UT. ..... uterine duct
DU.VIT. ..... vitelline duct
EX.P. ..... excretory pore
FOV.WAG. .. fovea Wageneri (ventral)
GA.A. ..... anterior ganglion
GA.P. ..... posterior ganglion
GEL. ..... gelatinous layer surrounding shell of egg
GL.PR. ..... prostate gland
INS.GEN. .... genital notch
M.R. ..... radial muscles of ejaculatory duct
NE.L. ..... longitudinal nerve trunk
OVC. ..... ovicapt
OOT. ..... oötype
OVAR. ..... ovary
PA. ..... proximal papilla of ejaculatory duct
P.EJ. ..... male genital pore (ventral)
P.VAG. ..... vaginal pore (dorsal)
RA.D. ..... posterodorsal ramus from the posterior ganglion
RA.M. ..... posterolateral ramus from the posterior ganglion
RA.V. ..... posteroventral ramus from the posterior ganglion
REC.OV. ... receptaculum ovorum
REC.SEM. ... receptaculum seminis
REC.VIT. .... yolk reservoir
ROS. ..... rosette
SH.GL. ..... shell gland
SP.AC. ..... acetabular spines
SP.AL. ..... anterolateral spines
TE. ..... testicular follicles
UT. ..... uterus
UT.P. ..... uterine pore (ventral)
UT.S. ..... uterine sac
VAG. ..... vagina (slender portion)
VAG.CR. .... vagina (thickened terminal section)
V.D. ..... vas deferens
VIT. ..... posterior limit of viteline follicles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GA.A.
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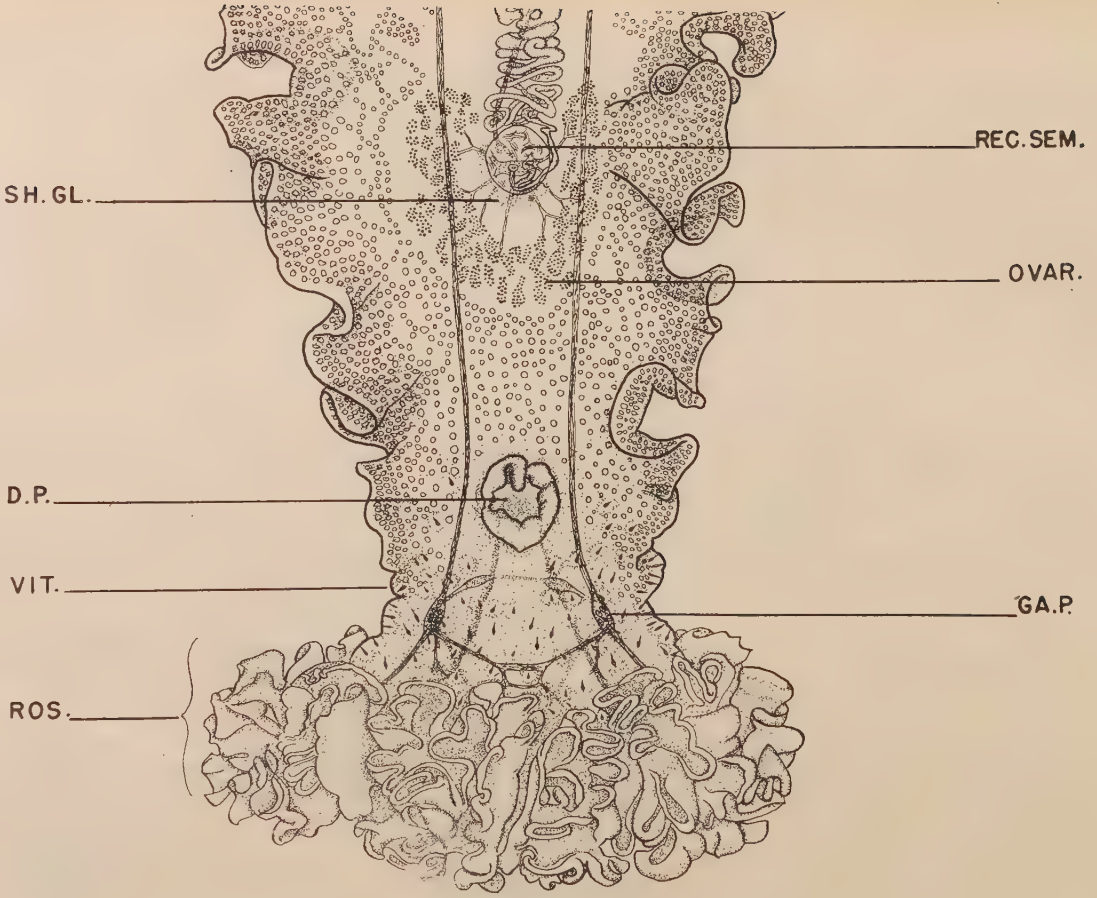


PLATE I

FIG. 1. *Gyrocotyle fimbriata*, dorsal aspect. ×8.6.

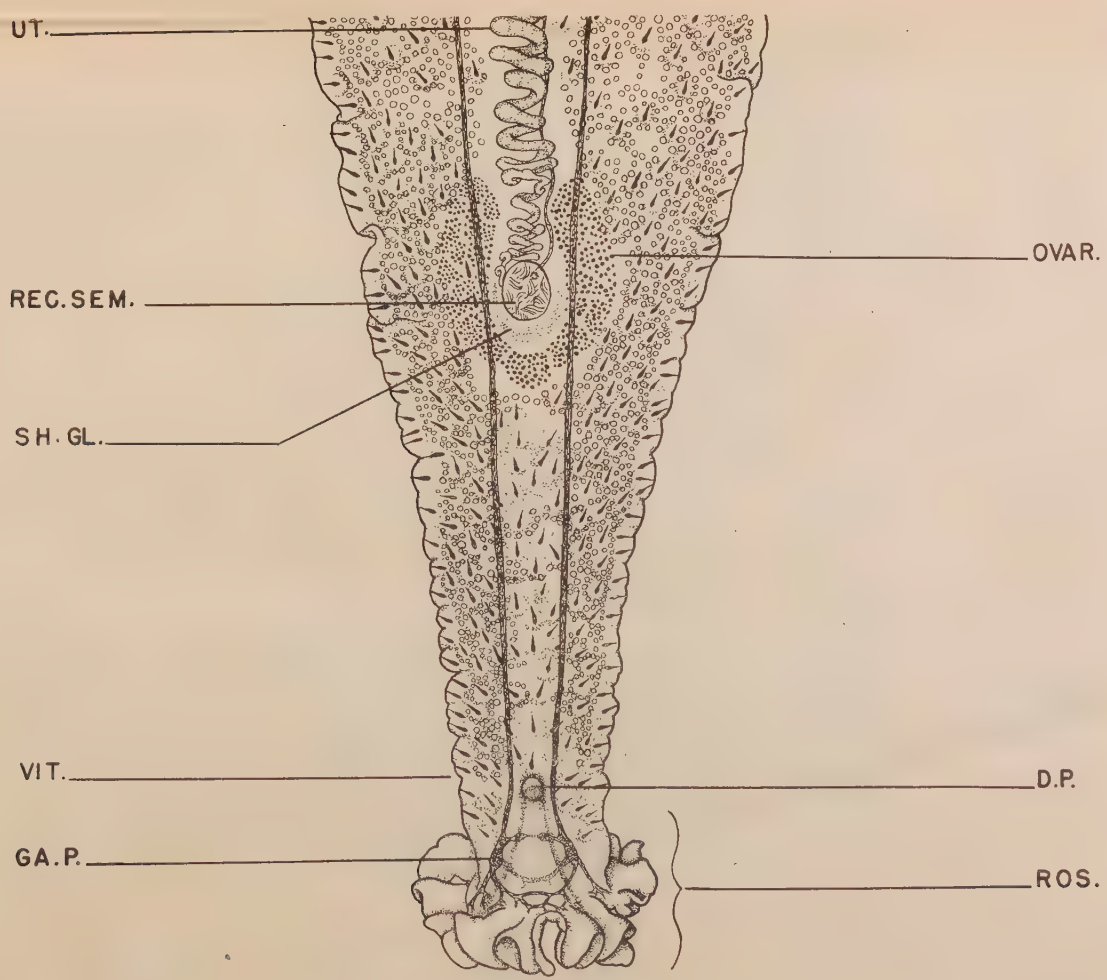
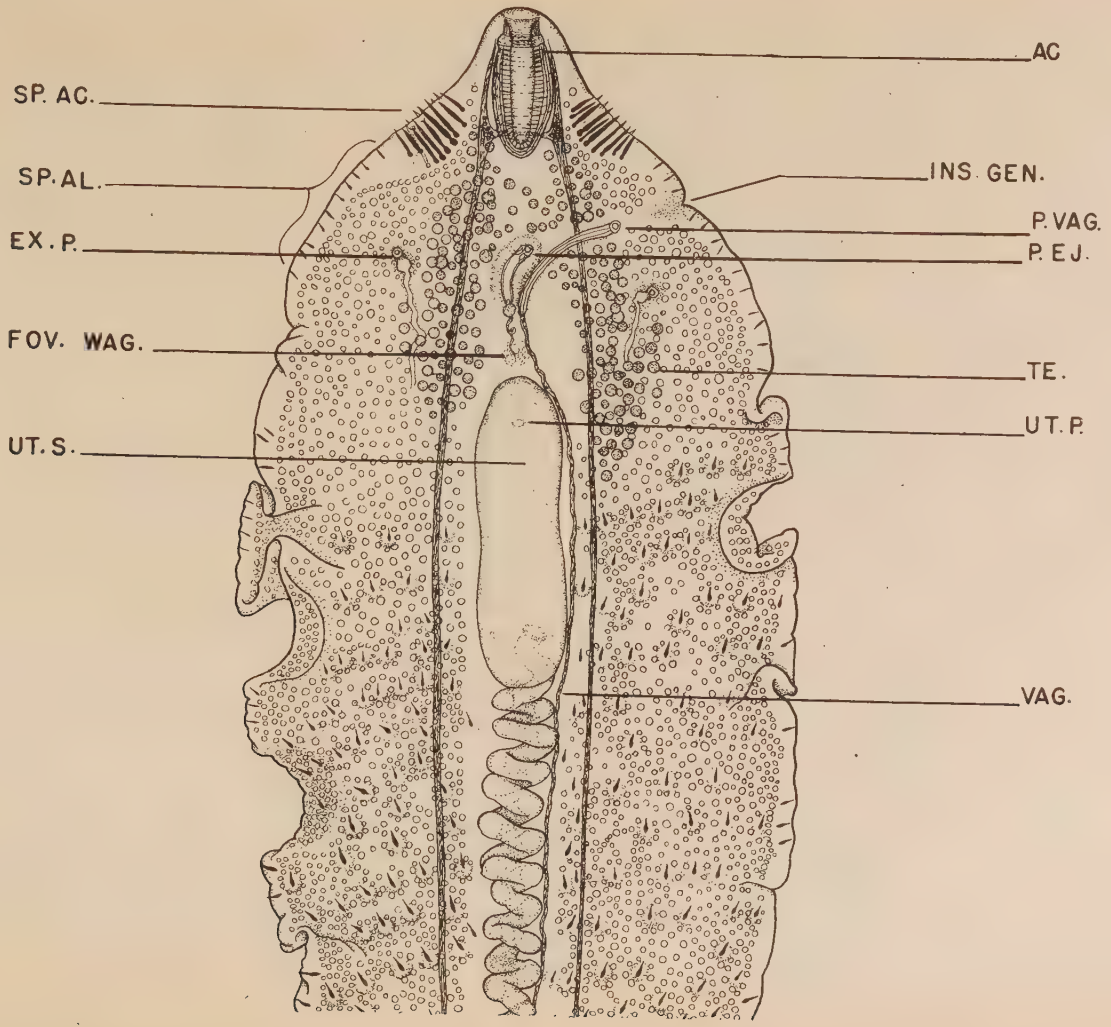


PLATE II

FIG. 2. *Gyrocotyle urna,* dorsal aspect. ×7.7.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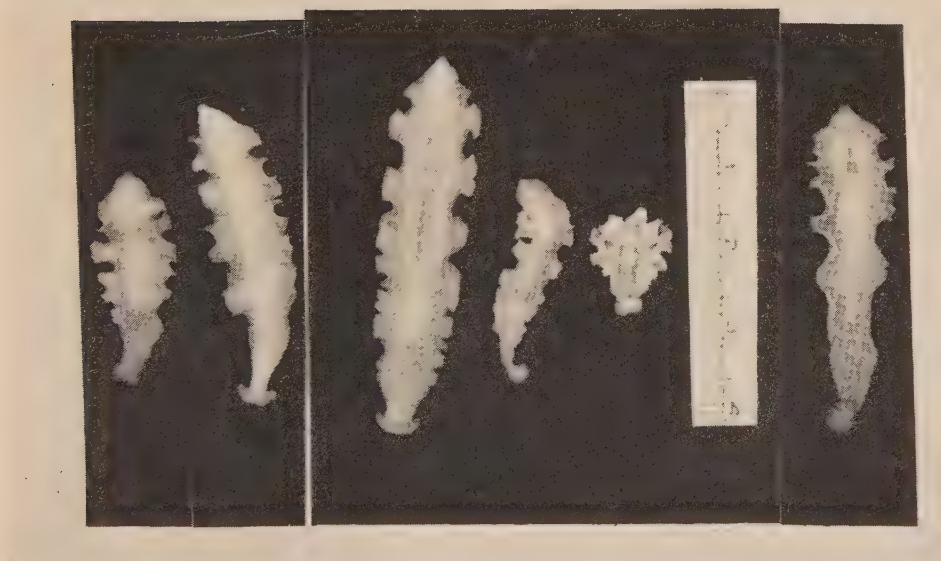


4.

PLATE III

FIG. 3. Preserved specimens of *Gyrocotyle fimbriata*. Photographs by Dr. K. Bonham.

FIG. 4. Preserved specimens of *Gyrocotyle urna*. Photographs by Dr. K. Bonham. In the second and third specimens from left, the papillae in which the spines are embedded are readily visible in the posterior third of the dorsal surface.

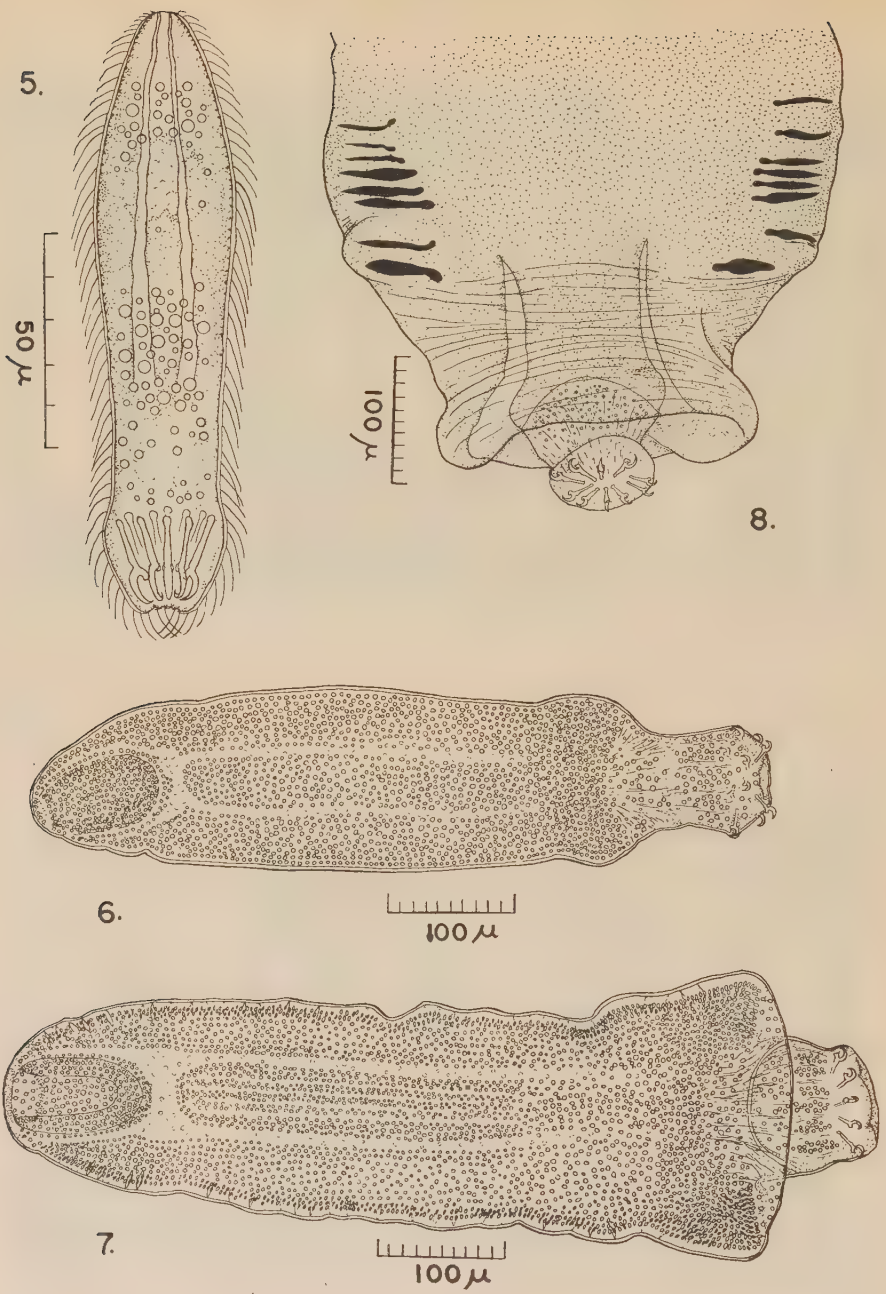


PLATE IV

FIG. 5. A decacanth larva of *Gyrocotyle fimbriata*. Drawing based on free-hand sketches of actively swimming larvae. ×568.

FIG. 6. Early postlarval stage of *G. fimbriata*, from the intestine of the ratfish. The swelling anterior to the hook-bearing papilla presumably represents the Anlage of the rosette. Drawing of a stained whole mount. ×168.

FIG. 7. Postlarval stage of *G. fimbriata*, from the intestine of a ratfish, showing early stage in the formation of the rosette. Drawing of a stained whole mount. ×168.

FIG. 8. Posterior end of a postlarval *G. fimbriata* 1.5 mm in length. Note early stage in rosette formation, completely circular, which surrounds the hook-bearing papilla. Drawing of a stained whole mount. ×168.

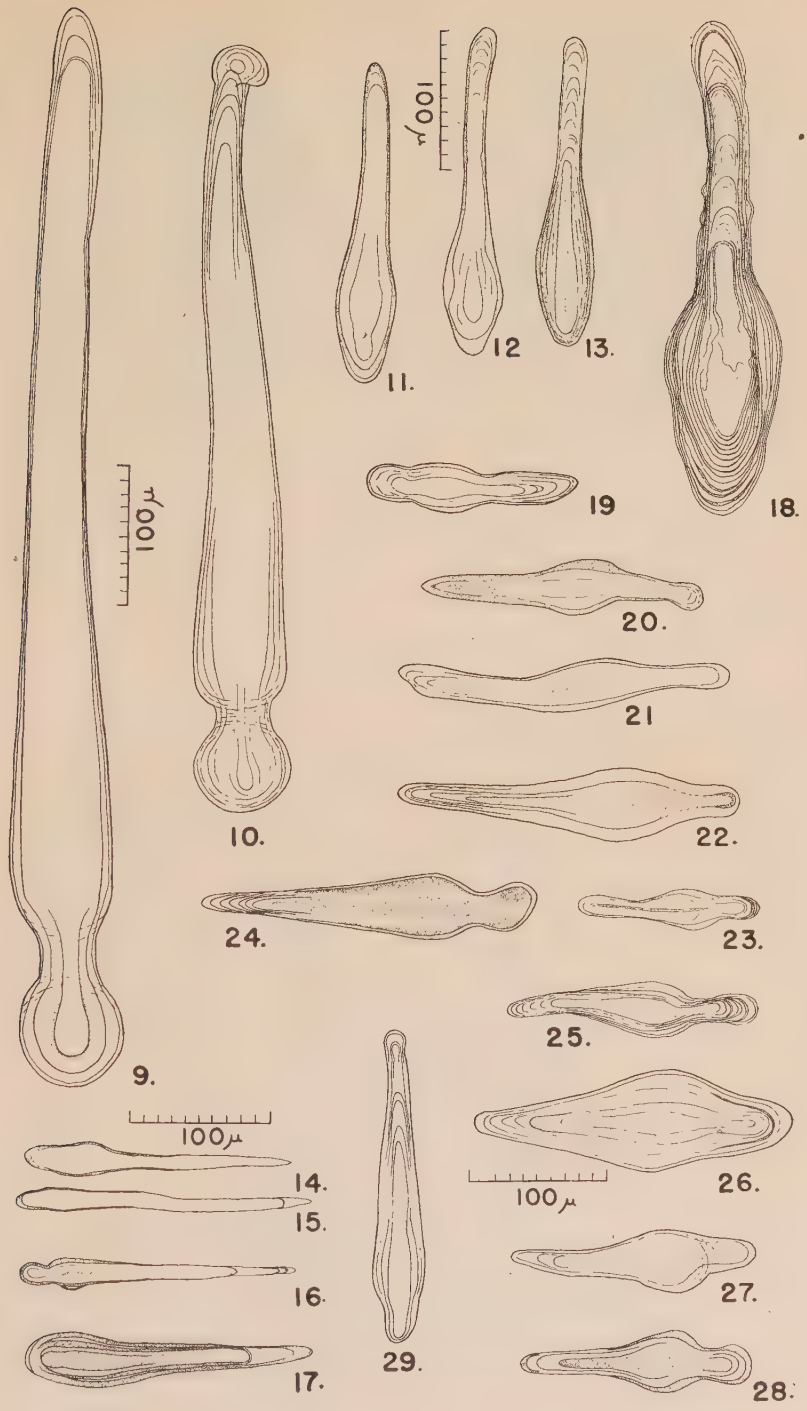


PLATE V

Figs. 10, 18 and 25 from specimens mounted in glycerine; the remainder from balsam mounts. All spines × 183.

FIGS. 9–10. Acetabular spines of *G. urna magnispinosa.*
FIGS. 11–13. Posterodorsal spines of *G. urna magnispinosa.*
FIGS. 14–17. Acetabular spines of *G. urna parvispinosa.*
FIG. 18. A posterodorsal spine of *G. urna parvispinosa.*
FIGS. 19–23. Acetabular spines of *G. fimbriata.*
FIGS. 24–29. Posterodorsal spines of *G. fimbriata.*

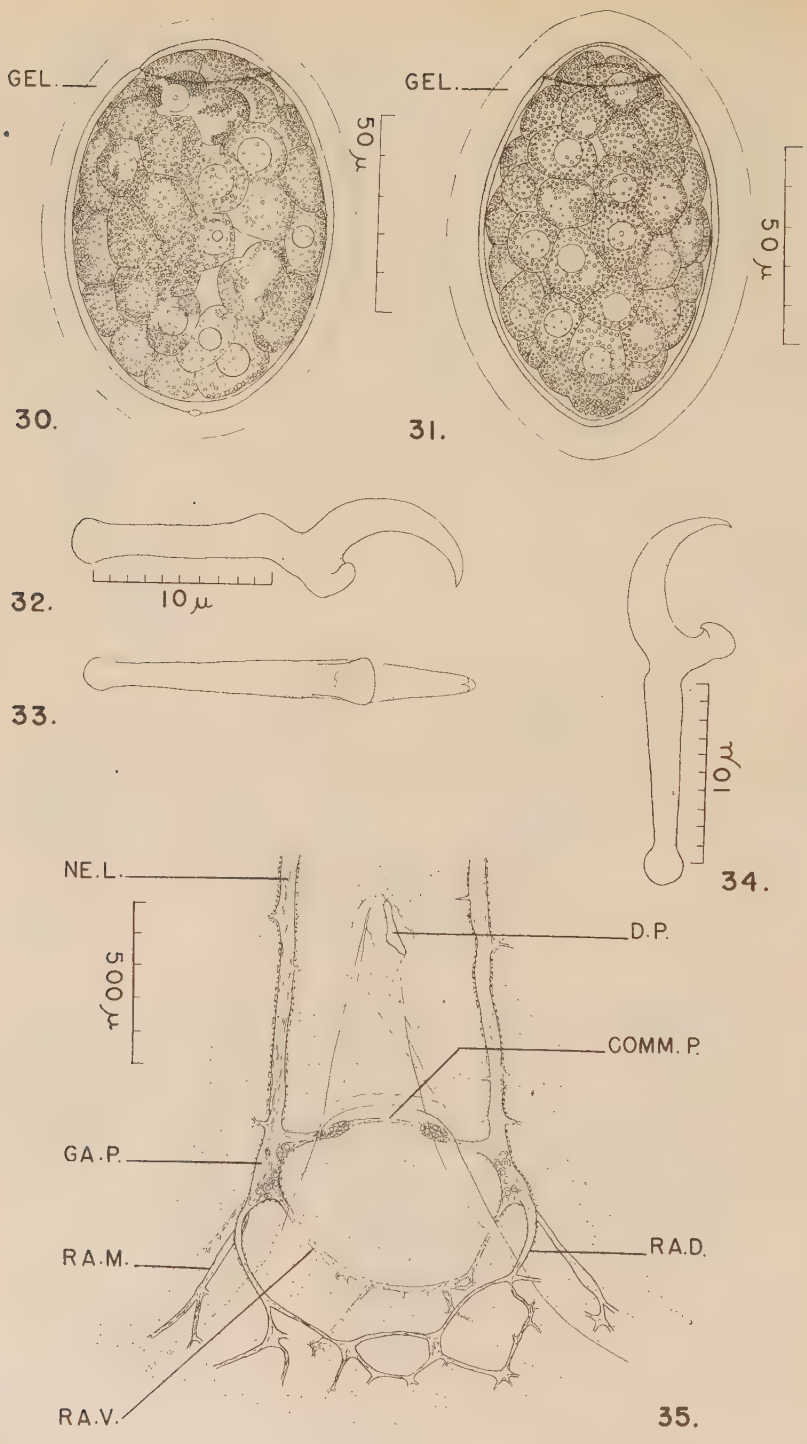


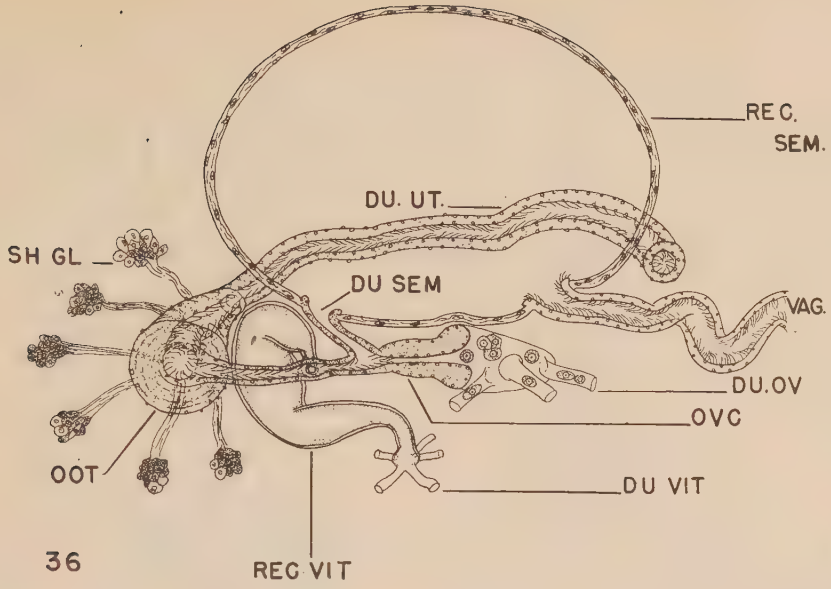
PLATE VI

FIG. 30. A spontaneously deposited egg of *G. fimbriata*. ×515.

FIG. 31. A spontaneously deposited egg of *G. urna*. ×515.

FIGS. 32–34. Hooks of the decacanth larva of *G. fimbriata*. Enlarged copies of camera lucida drawings. ×2375.

FIG. 35. *Gyrocotyle urna*. Posterior end of the nervous system between the dorsal pore and the funnel and the rosette. ×43.



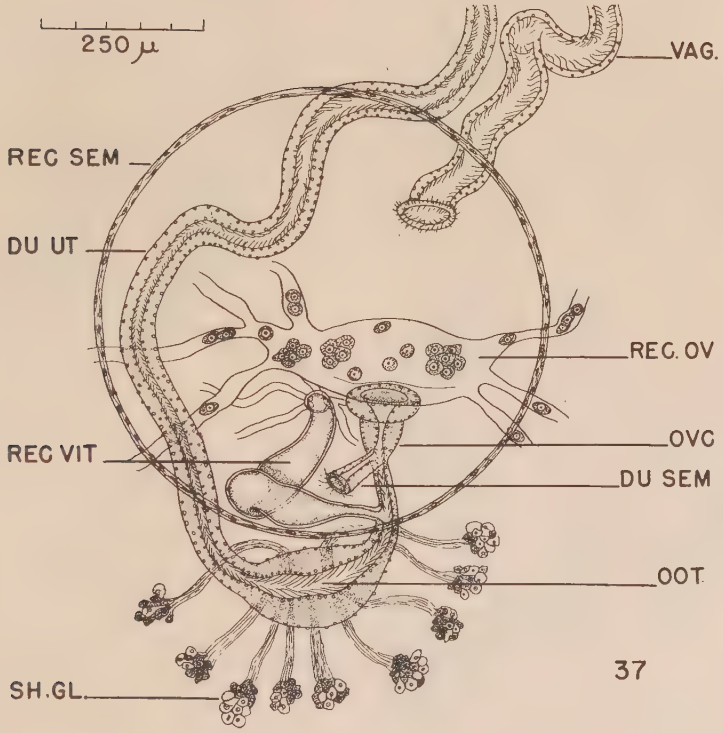


PLATE VII

FIG. 36. Junction of the ducts of the female reproductive system and the beginning of the uterus in *G. fimbriata*, right lateral aspect. Reconstructed from serial sections. ×85.

FIG. 37. The same, in dorsal view. ×85.

In the interest of clarity, Figs. 36 and 37 are schematized as follows: (1) The bladderlike receptaculum seminis is depicted in outline, only. (2) The vitelline ducts and reservoir, normally crowded with yolk cells, are represented as empty. (3) The follicles of the shell gland are much more numerous than depicted in the drawings. (4) The seminal duct, normally dorsal to and nearly parallel to the ovicapt, has had its proximal end shifted posteriorly.

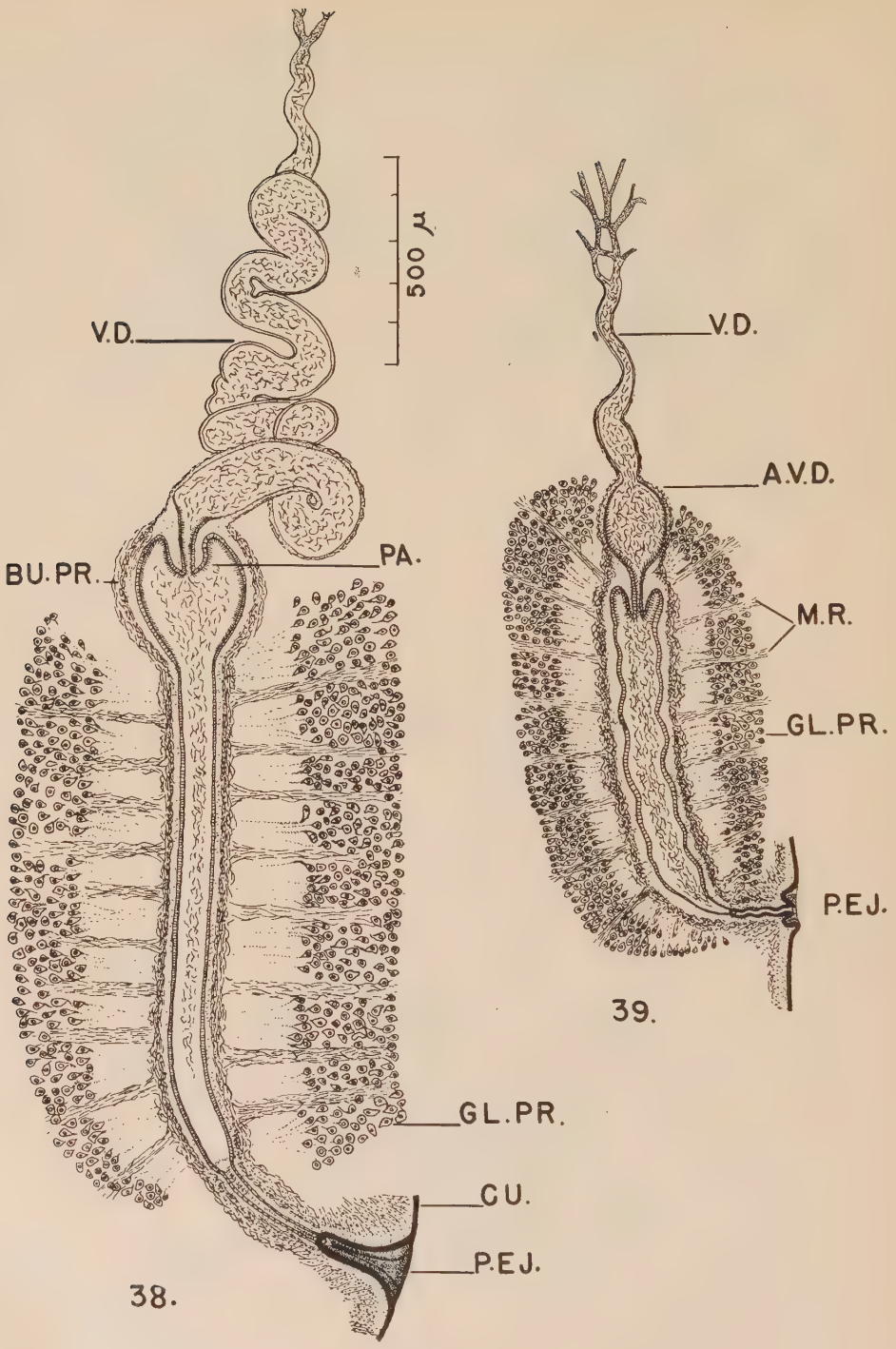


PLATE VIII

FIG. 38. Diagram of the ejaculatory duct and vas deferens in *G. fimbriata,* left lateral view. ×56.

FIG. 39. The same, of *G. urna.* ×56.

The magnification in Fgs. 38 and 39 is not sufficient to show histological detail. The outline of Fig. 38 was traced with a camera lucida from a thick sagittal section; Fig. 39 was reconstructed from serial sections.

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